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British universities must win lost friends

For British universities, the crisis of the past several years is about to reach another climax. Last week, the University Grants Committee sent off its promised letter, which emerged as a restatement of a now familiar problem. Next month, universities will be told individually what they will be able to count on from central government in the next three years. Then, with this academic year almost over, universities will know whether the prospect for the immediate future is a bad as they have been fearing these past several months, or not quite as bad. There is no chance that they will be pleasantly surprised, although it will be of some comfort that the government has now promised that the misery ordained to begin this summer will at least be assured for a full three years. At the end of that time, the British university system will be transformed. Almost certainly, it will be smaller. Nobody knows quite how.

The nature of the impending change is by now well understood. From the beginning of this academic year, the central government's support for universities has been decreased by the notional cost of educating overseas students, which the universities are expected to recover by charging economic fees. If the market for British higher education were insensitive to price, the financial consequences would be negligible. In practice, since unknown proportions of overseas students will now go elsewhere, a substantial amount of money will be taken out of the system. The consequence may be a reduction of up to seven per cent in the aggregate income of the universities, but the consequences will not be uniformly spread. Some universities will fare worse than others, but to a degree quite unrelated to their intrinsic worth. The British government has also decided that there should be a reduction in the resources made available to the universities. This will amount to just over 3 per cent next year, and to 8.5 per cent in three years. Throughout the university system, committees have been working hard, making lists of the steps they may have to take in the months ahead. Getting rid of academic departments, and of people, appears on most prospectuses. Shutting down whole universities has been canvassed as a possibility, but is unlikely.

Many universities and university teachers are bewildered by the abruptness of the change that has overtaken them. Gone for good are the happy 1960s, when both Conservative and Labour governments urged expansion (so as to increase the proportion of young people going on to higher education) and appeared willing to provide the means. What can have changed? And why has the change come so suddenly? Many academics are still plaintively asking these questions. The answer is that the change is not nearly as abrupt as it may seem. The climate has been changing since the early 1970s, since when public support for the universities and their research has stagnated. For most of that time, academics have been foolishly complacent, often behaving as if the deterioration of their institutions was the responsibility only of the central government, aided and abetted by the University Grants Committee. Now, the government can fall back on the thin excuse that further impoverishment is justified by the impending reduction of the age groups from which university students are recruited. The underlying truth, however, is that the British universities have lost their political friends. Many in the House of Commons will be sympathetic to the plight of the universities in the next few months, but few elected politicians would use up political capital by defending the universities from the cuts ahead.

The universities are not blameless. From time to time, they have almost courted unpopularity. Student troubles have not helped,

even though the British university has been less threatened by them than many others. Even now, however, minor issues concerning students affect the capacity of the universities to keep their political friends. Is it right, for example, that the cost of running students' unions, hitherto a charge on local authorities, should now be paid from the dwindling budget of the University Grants Committee? Why should not students pay? The more serious alienation of the universities from their traditional supporters in the House of Commons springs more directly from the assumption of the universities that even in times of rapid and painful social change, the structure of the university system itself should remain untouched, changing only if the universities so decide. One result is that even those outside the system who value academic freedom are asking whether academic freedom necessarily requires lifelong tenure for all. Then those among the universities' natural friends who accept that scholarship is an indispensable part of the institution of the university are unconvinced that the universities are vigilant enough in distinguishing good scholarship from the less good or even the downright bad. The universities have lost many potential friends by their unimaginative, even unhelpful, responses to several attempts to reform the transition in the British system from school to university. And their proper insistence that universities are self-governing institutions is weakened by their frequent demonstrable failure to manage their own affairs effectively.

Hard words such as these are not inappropriate even in hard times. Indeed, if the universities do not now acknowledge that they are partly responsible for the plight in which they find themselves, they will be less able to meet the crisis the British government has unthinkingly forced on them. They will, in particular, be less able to make the plain argument that the policy of retrenchment being forced on them is a foolish policy, innocent of clear objectives, marred by vindictiveness and almost calculated to ensure the British taxpayers get even less value for the diminished sums of their money to be spent on higher education in the next few years. For several years, it has been clear that the most urgent need in British higher education is for a reconsideration of what it is for, how it should be financed and how different kinds of institutions, universities included, should contribute. Probably it is now too late for that reappraisal to be carried out deliberately. But radical thinking by threatened institutions might still do wonders.

It would be a useful start if the universities would decide where they stand in relationship to each other and to comparable institutions, the polytechnics for example. In the past few months, it has been fashionable among the enemies of higher education to say that there are too many universities. That assertion is unverifiable, and probably untrue. But there are too many universities of the same kind, or too many universities striving after an idealized vision of what a university should be — perhaps a cross between Oxbridge and the Harvard Business School. All except Scottish universities insist that, for most students, three years of undergraduate teaching is sufficient for a first degree. All insist that scholarship is inseparable from teaching and, more literally, that there must be a full range of research programmes undertaken on the premises. Most of them are so balkanized internally, with power and responsibility delegated to separate departments and colleges, that academic policy is a kind of statistical amalgam of different people's inclinations. And most universities scorn most other kinds of higher education than their own — the British polytechnics or



liberal arts colleges on the pattern of the United States.

Breaking this log-jam of prejudice against change is desirable in itself but could also have practical consequences in the present crisis. Given the quality of their students and the scale of their research programmes, many universities would be better off if they could become liberal arts colleges in the best sense — providing students with a general education (which includes an education in science) without the encumbrance of in-house research programmes (which does not imply that academics need not be scholars). Other universities, by contrast, might make their way more successfully in the world by concentrating more vigorously on the kind of vocational education that has made institutions such as the Cranfield Institute of Technology (not strictly a part of the university system because it is financed directly from central government) both successful and valuable. British universities will be the more able to turn the tight corner ahead if they come to believe that diversity within the system should be an objective — and if the University Grants Committee can devise an incentive for helping them stay with such objectives.

Hotchpotch

The hotchpotch system of financing universities also needs reform. Money for day to day purposes reaches university treasurers from three principal sources — from the Department of Education and Science on the recommendation of the University Grants Committee, from local education authorities which pay the fees of all home students (and which are reimbursed by the Department of the Environment) and from individual students from overseas, who must pay their own tuition fees as they go along. But the local authorities are also required by law to provide many students with a maintenance grant calculated after allowing for parental income. Most universities have only a negligible income to call their own, although many of the Oxbridge colleges are still well-heeled. In most universities, tuition fees now exceed twenty per cent of direct subvention while maintenance grants paid from public funds to university students are probably close on forty per cent of direct income. The government seems for the time being to have given up its ambition to replace maintenance grants by loans, but the impending crisis is caused largely by its threatened reduction of the direct grant. But in this crisis, there must be room for a redistribution of these substantial funds. When the interest of students in their education is matched by the universities' anxiety to survive, however, there must be places where it would be in everybody's interest — even the government's — that funds were transferred from the maintenance grant to the general budgets of the universities. The simple way of doing this would be to require that students should work for the institutions that educate them, thus reducing general costs. British students might in the process lose their unique status as perpetual dependants, but the world would not thereby end.

Hardship

Academics must also be prepared for hardship. The impending crisis has already raised questions about security of tenure — and many universities are dangling the prospect of early retirement before their staffs. In the period ahead, however, the universities may not be able to afford this dubious luxury — and the government is unlikely to help. So academics may have to make do with less pay, at least in real terms. Will they? The Association of University Teachers is already up in arms at the cuts in prospect, and is holding out the hollow threat of a strike. The academic profession will, however, be further discredited if, after decades of high-sounding (and cogent) argument in defence of the university as a social institution, academics let universities collapse by insisting that national agreements on pay must apply to everybody. Indeed, they should go further than be flexible on pay and agree to be flexible about the work they do. Research, they rightly insist, is inseparable from the university; but so is teaching. And those who do less research must be prepared to do more teaching.

Such developments, even when designed to meet the immediate crisis, cannot easily be brought about. Few British universities

have mechanisms by which their academic members can agree on the allocation of car parking space, let alone on matters even of academic policy. Yet academics are understandably suspicious of ceding such responsibilities to the university administrators. So even when the going is good, too little is decided. At times like the present, when ingrained suspicions are heightened, paralysis tends to supervene. Yet the crisis requires that universities should be individually equipped to make decisions of two sharply different kinds.

First, there will have to be decisions about the academic qualities of individual academics. Whose research is worth continued support? Who might fairly take on more teaching? Who may have to be asked to leave? And which departments are more dispensable than others? Traditionally, academics have avoided such decisions because they are invidious. If they do so now, decisions will nevertheless be made but in an unseemly way — or by drawing lots. And the universities as well as unlucky academics will be the losers.

Second, universities need ways of deciding strategies for survival. Committees of academics are ill-equipped for such tasks, or even comprehensively to list the alternatives. (Courses of action unpalatable to some are rarely heard of.) The standard compromise is to appoint a committee with an external and supposedly independent chairman to suggest what might be done — the mechanism is now being followed by the University of London. The trouble is that the recommendations that follow have no force, and can be endlessly disputed. As things have turned out, there is no choice for the universities but to agree that their administrators have a duty to draw up the agenda for the inevitable debate on what course should be followed — and also a right to bring an end to argument. In reaching what must be painful decisions, the universities singly would benefit from some means of knowing that they are not all rushing off in the same direction but, rather, moving towards diversity.

Self-interest

The peculiar difficulty of the problems with which the universities are now confronted is that their own narrow self-interest may not coincide with the long-term interests of the university system as a whole or with the interests of their customers — in the last resort, their potential students and those prepared to sponsor students, ultimately the taxpayers. Plainly, however, it will serve no useful purpose if individual universities decide to concentrate on courses for which there is no demand, or on fields of research for which there are no sponsors. Up to a point, such market forces will keep individual universities moving in prudent directions. But the market for higher education is far from perfect — and should in everybody's interest remain so. It would be tragic, and a travesty of the idea of a university, if students' choices of courses were determined exclusively by short-term economic considerations, or if grant-making bodies made grants for research on the basis of expected communal benefit. Both students and the sponsors of research are known to make mistakes. And the social value of universities derives directly from their capacity to help devise an uncertain future.

These are the steps that British universities must take in the weeks immediately ahead. There is another course of action that they cannot neglect. The government's decision that there must be a cut in university funds has been plucked like a rabbit from a hat. The consequences have not apparently been considered except by those among the government's economists concerned with the size of the borrowing requirement. So far, both the University Grants Committee and the universities have been willing to administer this sudden change of policy without any but the mildest protests. Lacking friends, the universities may think they have no choice. But, in the long run, the present crisis will seriously damage the interests of a large part of the British population. It is time that the universities abandoned good manners, and told the government what will be the consequences of its action. And it is not too soon to set out to win back the lost constituency of friends, presumably first among parents whose children will, on present form, be deprived of higher education.

Creationism again an issue in Arkansas

Civil liberties take suit against law

Little Rock, Arkansas

The American Civil Liberties Union (ACLU) has decided to engage in a full-scale confrontation with the growing creationist movement over the teaching of evolution in US public schools. Early next month, ACLU is planning to file a complaint against the State of Arkansas arguing that a law passed by the state legislature in March — which requires that "creation-science" be given equal time with the theory of evolution whenever the latter is discussed in public school classrooms — is unconstitutional, since it ignores the required separation of church and state.

The ACLU case may well find its way up to the US Supreme Court. It seems certain to generate as much controversy as the last time that the organization confronted the creationist arguments. This was in 1928 when Clarence Darrow, acting as an ACLU volunteer attorney, defended Tennessee biology teacher John Scopes against the charge that he had violated state laws by teaching evolutionary theory in the classroom.

Although Scopes was convicted and fined \$100, Darrow won a moral victory — and, over the following decades, individual states removed from their statute books laws which prevented Darwinian evolution from being taught.

This time, however, the tables have been turned. The creationists have spent many years developing the argument that their ideas can be described as "scientific", and in eliminating all explicit religious references from public school textbooks promoting the idea that the world originated in the hands of a divine creator. More recently, they have spent an equal amount of legal effort in eliminating all explicit religious references from proposed legislation at both the state and the federal level, demanding equal time for what are described as two rival scientific theories.

The legislation introduced in Arkansas, for example, has been carefully crafted by a creationist group in South Carolina, with advice from sympathetic lawyers across the country, to answer precisely the type of challenge which ACLU is planning to mount.

Various other states have been discussing similar "equal time" bills over the past two years. Last year, for example, the Georgia legislature just failed to approve a bill in the closing minutes of its 1980 session. And according to Mr Paul

Ellwanger, head of the group in Anderson, South Carolina, which wrote the Arkansas bill (known as the Citizens for Fairness in Education), seventeen other states have been considering similar or identical bills in their current or recently-ended sessions.

Mr Ellwanger emphasizes that his own group is not formally linked to any political or religious organization. In Arkansas, however, two significant factors seem to have played a major role in getting the "equal time" legislation on to the statute book. The first was the important role played by groups such as the state branch of the Moral Majority and the Little-Rock-based organization Family, Life, America, God (FLAG) in stimulating support for conservative candidates — such as Arkansas' current governor, Mr Frank White — in last year's election. The second was the speed with which state lawmakers were prepared to push the bill through both houses of the legislature.

The bill was introduced into the Senate by Mr Jim Holsted, a first-term Senator from North Little Rock, a wealthy suburb of the state capital. He has admitted that he knows little about creation-science, but as a "born-again" Christian is a firm believer

in the book of Genesis. According to Holsted, he was given the bill in virtually completed form by a mathematics teacher at a Jacksonville High School, who had already been attempting to persuade the local school board to require "equal time" for creationism and evolution in the schools under its jurisdiction (a tactic which has already been successful in several states).

The bill was passed overwhelmingly on the floor of the state Senate. Several members of the Senate said later that, although they did not necessarily agree with the content of the bill, they had been reluctant to vote against it, since to have done so would have been interpreted as a vote against God — a heavy risk to take in a strongly Bible-oriented state.

The bill was introduced almost immediately into the House of Representatives by a business neighbour of Mr Holsted's, Representative Cliff Hoofman. It passed to the education committee, where the bill's supporters and opponents were each given five minutes to put their case — the sum total of public discussion of the bill's contents. A motion to delay the committee vote was refused by the

Arkansas legislation reflects old quarrels

The Arkansas law, passed in March (and introduced in virtually identical versions in seventeen other states), is known as the "Balanced Treatment for Creation-Science and Evolution-Science Act". Its principal requirement is that "public schools within this state shall give balanced treatment to creation-science and to evolution-science. Balanced treatment of these two models shall be given in classroom lectures and in textbook materials taken as a whole for each course, and in library materials and other educational programs".

"Creation-science" is defined as the scientific evidence for creation and inferences from that evidence. This includes evidence of: sudden creation of the Universe, energy and life from nothing; the insufficiency of mutation and natural selection in bringing about development of all living kinds from a single organism; changes only within fixed limits of originally-created kinds of plants and animals; separate ancestry for man and apes; explanation of the Earth's geology by catastrophism, including the occurrence of world-wide flood; and a relatively recent inception of the Earth and living kinds.

The bill contrasts this with the teaching of "evolution-science" — referred to earlier as the theory of evolution — and the evidence which is held to support it. Whereas previously both creation and evolution had been referred to as

"theories" to which equal time should be given in the science classroom, the bill passed in Arkansas refers to the two "scientific models" of evolution-science and creation-science; it also states as a "finding of fact" that "only evolution-science is presented to students in virtually all of those courses that discuss the theory of origins. Public schools generally censor creation-science and evidence contrary to evolution".

Particularly controversial has been a passage in the bill which states that one of its purposes is to prevent "establishment of theologically liberal, humanist, nontheist or atheist religions". The bill goes on to state that public school presentation of "evolution-science" is unconstitutional, because "it produces hostility toward many theistic religions and brings preference to technological liberalism, humanism, nontheistic religions and atheism, in that these religious faiths generally (*sic*) include a religious belief in evolution".

The Arkansas law also states, in a key passage designed to provide protection against the anticipated challenge, that: "public school presentation of both evolution-science and creation-science would not violate the constitution's prohibition against establishment of religion, because it would involve presentation of the scientific evidences and related inferences for each model rather than any religious instruction".

chairman, and after a short discussion it was passed and sent onto the floor of the House, where it was brought up and hurriedly passed in the closing hours of the 1981 session at the end of March.

Governor White lost no time in signing the bill, admitting later that he had not read it before doing so. His decision provoked a storm of protest from scientists in the state, most of whom were unaware of the bill until it had been passed into law. The Arkansas Academy of Sciences at its annual meeting passed a resolution urging that the law be rescinded, as did 300 faculty members of the University of Arkansas at Fayetteville, who complained that the act would hurt the university's reputation.

Governor White, however, has made it clear that he does not intend to take any steps to repeal the law unless it is judged as

unconstitutional. And many Little Rock observers feel that he is likely to stick to this position, given the extent to which his surprise victory over incumbent Democrat Governor Bill Clinton last November seems to have been helped by the active involvement of groups such as FLAG and the Moral Majority in encouraging the conservative vote.

As a result, the spotlight is now focused on the ACLU complaint, which both sides see as setting important precedents. Groups which are likely to support ACLU include the National Association of Biology Teachers, which has long campaigned about allowing creationist theories to be placed on the same level as evolution in school biology classes. And the President of the Arkansas Academy of Sciences, Professor John Kenneth Beadles, head of the State University of Arkansas' biology department, is suggesting to fellow academy members that they add their support to the ACLU effort.

The creationists are getting ready to meet the challenge. One of the principal advisers on the drafting of the Arkansas Bill is said to have been Wendell Bird, an attorney who was at one time an editor of the *Yale University Law Review*, and who now works with the Institute for Creation Research in San Diego, California. The institute, as a tax-exempt organization, takes pains not to be seen to be involved in legislative campaigns; however, Mr Bird's writings are referred to by Mr Ellwanger and others as powerful support for the argument that the teaching of creation-science can be defended by referring to rights guaranteed by the constitution.

If ACLU wins its case, either at the district court level, during the appeals process, or in the Supreme Court, it will be a major setback not only for the creationist movement — which now claims over 750 scientists among its ranks — but also for the broader political strategy of the conservative right. If it loses, it will be a significant boost to creationists, who are already predicting that bills similar to that passed in Arkansas are likely to be introduced in 40 state assemblies next year.

Pressure is also building up at a federal level. Several Congressmen have received a model bill promoting research support for "creation-science", from a group, also headed by Mr Ellwanger, called the Citizens Against Federal Establishment of Evolutionary Dogma. It seems only a matter of time before some congressman introduces the legislation.

The two sides are as far apart as ever. Mr Ellwanger argues that "fairness should not be an option; we now have the tools to insist that fairness be mandated by law, in the interests of academic honesty". The *Arkansas Gazette*, which has carried out an uncompromising campaign against the bill, warns that it will brand the state as a "bastion of know-nothingness in the teaching of science." The court case is unlikely to stay polite.

David Dickson

Sakharov conference

Birthday honour

New York

The two-day conference at Rockefeller University on 1–2 May in honour of Dr Andrei Sakharov was an ironic occasion. The opening day was celebrated in the Soviet Union with the customary package of approved slogans, the forty-first of which reads "Soviet scientists! Improve the effectiveness of research! May the alliance between creative thought and creative labour be much stronger!" Sakharov meanwhile remained in internal exile in Gor'ki.

The conference had been called in part to mark Sakharov's birthday on 21 May and consisted of three parts — Sakharov's work in science, for nuclear disarmament and for human rights. Among the scientific papers was one by John Archibald Wheeler (University of Texas) on Sakharov's "sausage-skin" cosmology, another by Harold Furth (Princeton) on Sakharov's contributions to fusion research and a third by Val Fitch (Princeton) on anti-matter asymmetry. Those present nostalgically recalled Sakharov's involvement with the international scientific community in the past twenty years and argued that his present isolation was tantamount to bringing his scientific career to an end.

The conference was at something of a loss to reconcile Sakharov's work on the Soviet thermonuclear bomb in the 1950s with his more recent concern for disarmament; the argument that by assisting in the strategic parity between East and West he was helping to satisfy one of the realistic preconditions of disarmament was regarded by some as artificial and by others as unnecessary.

Sakharov's own message to the conference (see page 184) was largely concerned with human rights and, in particular, with the plight of Soviet citizens other than himself. On the question of the relationship between professional scientists in the West and their Soviet colleagues, Antonino Zichichi, president of the European Physical Society, told the conference that more than a thousand scientists had undertaken that until the end of this year, all scientific papers submitted for publication would bear the legend "In honour of the 60th birthday of Andrei Dmitrevich Sakharov". For the time being, however, the disputed question of a scientific boycott of the Soviet Union such as that organized last year, although aired at the conference, has been allowed to lapse.

Another of the ironies of the timing of this month's conference is that it followed soon after the celebration of the twenty-fifth anniversary of the Joint Nuclear Research Institute at Dubna, south of Moscow. In a full-page feature in the daily newspaper *Sotsialisticheskaya Industriya*, attention was drawn to the search for

Scopes again?

The present is not the first occasion when Arkansas has been the focus of national attention on the constitutionality of efforts to influence the teaching of evolution in public schools. In 1968 the US Supreme Court struck down a law, introduced into the state in 1928, which made it illegal for any teacher and educational institution supported by state funds to teach "the theory or doctrine that mankind ascended from a lower order of animals".

Following the Scopes trial, no serious attempts seem to have been made to enforce this law. However, the case was passed to the Supreme Court after the statute had been unsuccessfully challenged in the state's Supreme Court by a biology teacher in Little Rock's Central High School, Mrs John O. Epperson.

In declaring the state law unconstitutional, Justice Abe Fortas argued that the 1928 law, enacted in a popular initiative, was a clear effort inspired by the Scopes trial to outlaw all but the "Bible story" of creation. "There can be no doubt that Arkansas has sought to prevent its teachers from discussing the theory of evolution because it is contrary to the belief of some that the Book of Genesis must be the exclusive source of doctrine as the origin of man" he said.

At the time of the trial, Arkansas and Mississippi were the only states to maintain an anti-evolution law. The fact that the law preventing the teaching of evolution was judged by the Supreme Court to infringe the "establishment of religion" clause in the constitution is expected to be used by the American Civil Liberties Union in its attack on the new law. Scopes himself, then living in Louisiana, hailed the Epperson decision as "what I have been working for all along". His own conviction was quashed by an appeals court, so he was unable to challenge the constitutionality of the Tennessee law.

transuranic elements in the "island of stability" (atomic numbers 114–120), studies of black holes and quarks. Mr Leonid Brezhnev, at the twenty-sixth Party congress, had emphasized the need for "fruitful cooperation of all states" on these problems, yet Sakharov has been unable to meet or correspond with his colleagues abroad since 22 January 1980.

Vera Rich

UK industrial research

All change

Britain's Department of Industry has reorganized the way in which it supports industrial research, seemingly to counter criticism that it has been poor at defining priorities and has relied too heavily on reacting to research proposals from industry. The nine research and development requirements boards, each aimed at a particular sector of industry, have been replaced by five boards, each dealing with a particular technology.

The five new boards are to cover the same spread of research as the old ones, the idea being that by increasing the spread of each board's responsibilities the government's definition of long-term objectives and priorities will be made easier. Hence for example, the new Mechanical and Electrical Engineering board will take over the work of the old Ship and Marine, Mechanical Engineering and Machine Tools and Electrical Technology boards. The old Chief Scientist's board, which considered topics not covered by the other boards, is to be incorporated into the new Textile and Other Manufactures board.

The other new boards are Electronics and Avionics which replaces Computer Systems and Electronics, Materials and Chemicals which replaces Chemicals and Minerals and Engineering Materials, and Metrology and Standards which remains the same under the chairmanship of Dr Duncan Davies, now called Chief Engineer and Scientist in the department.

The functions of the new boards will remain largely unchanged. Industry and universities will be able to apply to them for government support for industrial research and they will continue to place contracts for research of their own choosing within industry and the department's own research establishments. The arrangement whereby levies are charged on the profits accruing from research funded by more than 50 per cent by the department will remain. And each board will have an executive committee to approve projects. It is hoped that the reorganization will encourage the boards to place more contracts in priority areas of research. However, the fact that there are no plans to appoint subcommittees has led to some fears that the boards' remit could be too wide to be effective.

The department's budget for industrial

research is to remain roughly constant. This year it is £600 million, £35 million of which will be spent in industry and £25 million in the department's research establishments. A small saving in funds will be made from the reduction in staff expected to follow the reorganization.

Judy Redfearn

Bioengineering hazards

Europe doubts

Brussels

An inconclusive meeting on the safety aspects of research on recombinant DNA was held here last week by the European Community's Economic and Social Committee (ESC). At this stage, it is hard to tell whether the meeting will influence the development of Community legislation on the subject — and, if so, in what direction that influence will be directed.

The discussions ranged from the long-term ethical problems posed by the possibility of tampering with human genes to the risks of accidentally releasing a new and dangerous strain of microorganism. The impetus for the meeting was the European Commission's draft recommendation on the registration of DNA research — ESC is required to give an opinion before the Council of Ministers make a decision.

Most participants seemed to agree that industry was already being over-cautious. By trying to avoid the mistakes of the nuclear industry, the safety measures recommended in the early 1970s for DNA research now seem to reflect exaggerated fears.

One pointer in this direction was the conclusion by the European Science Foundation's Liaison Committee on Recombinant DNA in January that recombinant DNA research as such entails no significant novel biohazards. It was also stressed that recombinant DNA techniques can be safer than conventional methods for studying pathogens and toxins. Safety guidelines for handling pathogenic viruses, it was argued, were far more necessary, and the World Health Organization's guidelines are late in arriving. These are due to be published within the next few months.

It was over the social risks that most of the participants were divided. Representatives of trades unions pointed out that there was a danger that decision-making would be restricted to industry. British speakers pointed out that the British Genetic Manipulation Advisory Group has retained control, but French participants conceded that this was not the case in France and elsewhere. Several participants were convinced that the concentration of knowledge in the hands of industry will lead to commercial (or even military) applications not necessarily beneficial. Calls for less secrecy were, however, answered by reference to the fact that all patents are by definition publicly available.

But does the public have sufficient say

over the uses to which the recombinant DNA techniques will be put? This issue overlapped with that of the role of the media in informing the public. By attempting to bridge the gap between the scientist and the layman, was there a danger that the press was exaggerating the dangers or speed of developments in this field?

Although many of the speakers argued that the DNA guidelines were safety regulations looking for a hazard, some members of ESC confirmed afterwards that they were in favour of legally enforced safety regulations, even if they needed to be frequently revised to take into account changes in risk assessment. It was suggested that the Community was merely going through the same process that had taken place in the United States a few years ago, but a little later because of the several countries involved.

Jasper Becker

US defence budget

Laser destruction

Washington

The US Senate last week proposed adding an extra \$50 million to the budget of the Department of Defense to speed up the research and development of a space-based laser system designed to shoot down incoming enemy missiles. At the same time, a Senate appropriations committee has not passed on Mr Reagan's request for \$20 million to be added to the Pentagon's construction funds for the current year to begin work on the production of binary chemicals weapons at the Pine Bluff arsenal in Arkansas, although this decision could be overturned in negotiations with the House of Representatives, which has already given its approval for the money.

The decision to add additional funds for the laser system is the result of growing pressure from the US military to respond to reports that the Soviet Union is already testing ways of using lasers to destroy missiles — and in the face of continuing scepticism from several quarters that space-based lasers can in fact be produced with the required degree of reliability and accuracy.

The proposed \$50 million, which will include a detailed systems definition of a space-based laser weapons system, is a compromise between the \$250 million being requested by some advocates of the system, and fiscal conservatives concerned about an unnecessarily large expansion of the arms budget, which the Senate approved at a record figure of \$136,500 million for 1982.

Asked for comments on the original \$250 million proposal, Dr John Foster of the Department of Defense's Defense Science Board said it was too soon to accelerate space-based laser development towards integrated space demonstration for any mission, and that "a push toward integrated on-orbit demonstrations would seriously endanger necessary [research and

development] work". However, according to Senator John Warner, the Defense Science Board has concluded that the potential utility of space-based lasers is significant and that an average of \$50 million per year should be added to the programme.

The money for the binary chemical weapons facility which the Senate armed services appropriations subcommittee has left out of the supplemental request for the fiscal year 1981 would have been the first step towards renewed chemical weapons production which has been halted since 1968. The Administration is thought to be moving cautiously, aware of the public concern about the deployment of a new generation of chemical weapons in Europe. The main debate will probably take place later this year, when the details of next year's budget request, likely to contain substantially more funds for chemical weapons, are debated in Congress.

David Dickson

Soviet space

Romanian resources

Last Friday's launch of Soyuz-40, carrying into orbit Romanian cosmonaut-researcher Dumitru Prunariu for a week's stay aboard the Salyut-6 space station, completes the series of manned flights envisaged under the Comecon "Interkosmos" programme. Since the series was inaugurated with the flight of the Czech Vladimir Remek in March 1978, these "international" programmes have taken on a standard form, regularly including Earth-resources photography, astrophysics, biomedical studies and technological experiments. On each occasion there has been a smelting and crystallization experiment, using a Soviet "Splay" furnace and a capsule provided by the non-Soviet partner, with an appropriate patriotic code-name: "Altai" for Mongolia, "Berolina" for East Germany and in the latest case, "Dacia" for Romania.

Each joint programme, however, has also reflected the contribution made by the non-Soviet partner to the whole "Interkosmos" programme. In the case of Romania, the emphasis was initially on instrumentation. According to Dr Ioan Ursu, First Deputy Chairman of the Romanian National Council for Science and Technology, from the beginning of the programme in the late 1960s, Romania had specialized in the production of magnetometers, specially adapted spectrometers and radiation detectors. Romanian instruments, he said, had contributed considerably to the accumulation of data on the chemical composition of the upper atmosphere, variations of the Earth's magnetic field and particle physics. The current programme, according to Dr Eugen Mendescu, Secretary of the Romanian commission for

space activity, will include the "Astro" experiment, which will attempt to capture super-heavy nuclei. Other innovations include an experiment on the behaviour of liquids in "microgravitational" conditions and the monitoring of dynamic disturbance of the space station itself, in order to determine possible effects on the experiments (the Polish "Syrena" crystallization experiments in 1978 seem to have run into trouble on this account).

What prospects the Soviet manned space programme now holds for international cooperation remains a mystery. Current plans for Soyuz-Salyut still include flights by a French and an Indian cosmonaut, and during last month's celebrations of the twentieth anniversary of Yuri Gagarin's flight it was suggested that Finland might also be invited to participate.

Future international cooperation may well have received a setback with the death last autumn of Academician Boris Petrov, head of the "Interkosmos" programme. Academician Petrov was a firm supporter of such international flights, unlike, for example, the President of the Soviet Academy of Sciences, Anatolii Aleksandrov, who on one occasion commented sourly that "Solyuz is not a trolleybus". Nevertheless, the options remain open. At a press conference in Berlin last month, cosmonauts Pavel Popovich and Vladimir Aksenov observed that a manned flight to Mars was a "problem for the coming decades" which could "best be settled" by means of international cooperation involving many countries.

Vera Rich

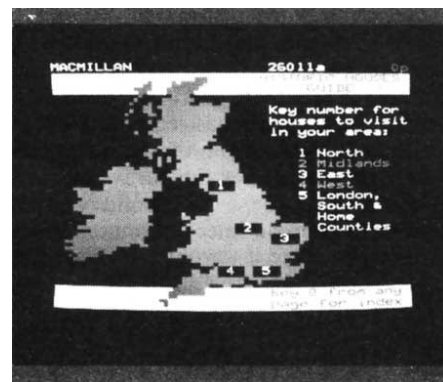
Viewdata systems

Battle joined

Although the British viewdata system, Prestel, was not expected to make a profit within the first eighteen months of operation, the results so far are disappointing. As yet only 1,288 people have bought the special television sets for domestic use. British Telecom's early hope that no home could do without its Prestel set seems to have been ill-founded. Sales to businesses, however, have been slightly brisker, although the 8,216 registered represent only a modest achievement.

British Telecom has, nevertheless, changed its marketing strategy, aiming at business rather than the domestic users. The National Economic Development Office hopes that business registrations will rise to 46,000 in the next eighteen months. The target for home registrations within that time is now 4,000.

The main lesson so far is that cost and the quality of information on the system are crucial to the home buyer. A custom-built Prestel set costs £400-500 more than an ordinary colour television set, although special adaptors costing about £200 are now coming onto the market. As well as ordinary telephone charges, a tariff for



connection to a central computer is also levied, so the cost to home users who want only information on train timetables, local cinemas and so on can seem expensive. The recent decision to increase the computer connection charge for peak day-time use does not augur well.

The quality of information at present stored on Prestel is variable. As yet nobody quite knows what type of information the domestic user is prepared to pay for, so two television supply companies are conducting a market survey in four provincial towns. Businesses, however, seem more prepared to pay for information, the greatest users so far being travel agents and financial companies.

One obstacle to further adoption by business is the increasing number of private viewdata systems, most of which are nevertheless compatible with Prestel. British Telecom regards these systems as complementary rather than competitive. It also hopes that the introduction next year of its Gateway system, whereby private databases can be linked to Prestel, will at least prevent British Telecom from being squeezed out of the market.

The teething problems of Prestel, the longest running public viewdata system in the world, will no doubt be watched with interest by competitors and potential buyers outside Britain. Although Prestel has been licensed to more telecommunications authorities than any of its rivals, the French Teletel and the Canadian Telidon may yet emerge on top. Arguments over which system should be recognized as a standard by the International Consultative Committee on Telephones and Telegraphs seem to have been resolved by all three being accepted. And the problem that no one system is compatible with either of the other two seems to have been resolved, at least for the French and British systems, by recent developments in software which will soon allow access by each system to the same computer.

Nevertheless, Telidon, which uses an alpha-numerical rather than an alpha-mosaic standard and is thus better at graphics, may have advantages over Prestel for some uses. What everyone wants to know is which system, if any, AT&T will choose in the United States. Some indication is expected to be given this week at Videotex '81 in Toronto. **Judy Redfearn**

Coal liquefaction

Trial by post

The future of the United Kingdom's only serious programme in coal liquefaction technology has been placed in doubt by a tense exchange of letters between the chairman of the National Coal Board (NCB), Sir Derek Ezra, and the Chief Scientist of the Department of Energy, Dr Tony Challis.

NCB has been waiting a year for a reply from the department about its proposal to build a 25-tonne-a-day pilot liquefaction plant at the Point of Ayr in North Wales, using two alternative technologies and costing £65 million to the government. These plans have become part of an agreement between the department, NCB and the National Union of Miners.

But, claims Dr Challis in his letter, on the assumption that oil conservation measures take full effect, and that sufficient oil-fired boilers are converted to coal-burning, "it seems likely that the United Kingdom can maintain net self-sufficiency in transport fuels and chemical feedstocks well into the next century", and thus that the United Kingdom does not need to develop an indigenous coal liquefaction technology, particularly when British coal is expensive (relative to American and Australian coal) and "bearing in mind the formidable level of international effort already applied in this field".

Dr Challis admits that NCB technology could be 30 per cent more energy-efficient than rival processes — after all, his own staff collaborated in the calculation — but says this is not enough to counter the 6-year lead-time in scale enjoyed by, say, the 200-tonne-a-day Exxon Donor Solvent plant at Baytown, United States. Dr Challis's letter, was written just after his return from a visit to this plant, so it seems Exxon have successfully "sold" him their technology.

He also argues that a 1-tonne-a-day plant followed by a 200-tonne-a-day plant would make better sense than the intermediate 25-tonne-a-day liquefier planned by NCB. Also, according to Dr Challis, who was a chemical engineer himself, NCB has "lacked adequate strength or perspective in the translation from chemistry to chemical engineering", and the translation has been done in too much of a hurry.

Sir Derek Ezra, in reply to Dr Challis, chides him for the department's late change of heart — and expresses surprise at Challis's optimistic view of Britain's oil supply, "more optimistic than the official view of the Department of Energy". And it is not at all clear, says Sir Derek, that countries with cheap surplus coal would be prepared to supply it cheaply in the future, when coal is in greater demand — so the cost of our own coal is not relevant.

Sir Derek believes that liquefaction technology will be needed in the United

Kingdom in the 1990s, and that NCB technology presents "a sufficient advance over the technologies being developed in the United States and Germany to merit support".

The American technology — and a similar plant under construction at Bottrop in West Germany — is, in fact, based largely on the wartime German technology (also employed at SASOL in South Africa) which splits the coal into monocarbon compounds, resynthesizing them to make light liquids. The resulting liquids possess only 30 per cent or so of the chemical energy of the original coal, whereas the NCB process which takes coal directly to light liquids, can promise 60–70 per cent efficiency.

British Petroleum is prepared to back the project, while NCB itself will contribute £18 million, and the project would receive about £12 million from the energy research fund of the European Community, if only the Department of Energy would give it their approval. Moreover, potential buyers of the technology in Canada and Australia will only show serious interest when a pilot plant is built.

However, despite Dr Challis's views, the

department has not yet made a formal decision about the plant. The next step should be a meeting shortly between the minister for coal John Moore, Sir Derek Ezra, Dr Challis and the research director of NCB, Dr Joe Gibson. If they can reach agreement, their recommendation will then be forwarded to the Secretary of State for Energy for a decision.

Meanwhile, in the United States President Reagan has appointed an Oklahoma businessman, Mr Edward E. Noble, to be chairman of the Synthetic Fuels Corporation which will oversee synfuel development in the United States. Mr Noble is reported to be uneasy at the large numbers of US technologies aimed at producing gas, and uncertain that the mandated targets of 0.5 million barrels a day (oil equivalent) of synfuel by 1987 and 2 million by 1992 can be reached.

In London, the director of the International Energy Agency said that given uncertainties over nuclear and renewable sources of energy, coal was poised to become the "single most important energy contribution to the future of modern society".

Robert Walgate

From rank weeds to riches

New Delhi

Workers in India have found a way of converting water hyacinth (*Eichornia crassipes*) into paper, and in so doing may have solved one of the Third World's major problems. This free-floating and fast-growing weed is not only a menace to navigation and hydroelectric power generation in affected countries, but also offers a breeding ground for pests and insects. So far, all efforts have been directed at eradication of the weed. Now, the Regional Research Laboratory (RRL) in Hyderabad, controlled by the Council of Scientific and Industrial Research, is making writing paper, posters, even invitation cards, using the leaves of water hyacinth collected from the city's lakes. Because the raw material is virtually free the paper is cheap and may save some of the trees cut down to feed the paper industry.

The technology for converting the weed into paper was developed through an international project initiated in 1978 by the London-based Commonwealth Science Council. The council, which identified water hyacinth as a major problem in the Third World, asked India to coordinate a project on the control and utilization of the weed and helped the collaborating countries to obtain a grant of \$200,000 from the United Nations Development Programme. While Sri Lanka, Bangladesh and Malaysia worked on biological control and biogas production, India chose to investigate the use of the weed in paper making. And, when scientists from the collaborating



Water hyacinth — paper power?

countries recently visited the RRL pilot plant, they were so impressed that they now plan to set up similar plants in their own countries.

The emergence of water hyacinth as a source of raw material for the paper industry should have great impact in India, where at least four million hectares of water surface are covered with the weed. The average yield of water hyacinth is 50 tonnes per hectare, giving India 200 million tonnes of non-forest raw material for paper. If even half of this is used, and assuming a 10 per cent conversion efficiency, the country can provide 10 million tonnes of paper from the weed. The new paper-making factories can be set up wherever water hyacinth grows and the process is such that the paper can be hand-produced by unskilled workers.

K.S. Jayaraman

The responsibility of scientists

from Andrei Sakharov

Because of the international nature of our profession, scientists form the one real worldwide community which exists today. There is no doubt about this with respect to the substance of science: Schrodinger's equation and the formula $e = mc^2$ are equally valid on all continents. But the integration of the scientific community has inevitably progressed beyond narrow professional interests and now embraces a broad range of universal issues, including ethical questions. And I believe this trend should and will continue.

Scientists, engineers and other specialists derive from their professional knowledge and the advantages of their occupations a broad and deep understanding of the potential benefits — but also the risks — entailed in the application of science and technology. They also develop an awareness of the positive and negative tendencies of progress generally, and its possible consequences.

Colossal opportunities exist for the application of recent advances in physics, chemistry and biochemistry; technology and engineering; computer science; medicine and genetics; physiology and hygiene; microbiology (including industrial microbiology); industrial and agricultural management techniques; psychology; and other exact and social sciences. And we can anticipate more achievements to come. We all share the responsibility to work for the full realization of the results of scientific research in a world where most people's lives have become more difficult, where so many are threatened by hunger, premature illness and untimely death.

The dangers of progress

But scientists and scholars cannot fail to think about the dangers stemming from uncontrolled progress, from unregulated industrial development, and especially from military applications of scientific achievements. There has been public discussion of topics related to scientific progress: nuclear power; the population explosion; genetic engineering; regulation of industry to protect the environment; protection of air quality, of flora and fauna, and of rivers, lakes, sea and oceans; the impact of mass media. Unfortunately, despite the urgent and serious nature of the issues at stake, such discussions are often uninformed, prejudiced or politicized, and sometimes simply dishonest. Experts, therefore, are under an obligation to subject these problems to unbiased and searching examination, making all socially significant information available to the public in direct, first-hand form, and not just in filtered versions. The discussion of nuclear power, a subject of prime importance, is an instructive example. I have expressed elsewhere my opinion that the

dangers of nuclear power have been exaggerated in the West, and that such distortion is harmful.

With some important exceptions (primarily affecting totalitarian countries), scientists are not only better informed than the average person, but also strive for and enjoy more independence and freedom. Freedom, however, always entails responsibility. Scientists and other experts already influence or have the capacity to influence public opinion and their governments. (That influence should not be exaggerated, but it is substantial.) My view of the situation of scientists in the contemporary world has convinced me that they have special professional and social responsibilities. It is often difficult to separate one from the other — the communication of information, the popularization of scientific knowledge, and the publication of endorsements or warnings are examples of activities with both professional and social aspects.

Similar complications arise when scientists become involved in questions of disarmament: in developing strategy for or participating in international negotiations; in advancing proposals or issuing appeals to governments or to the public; and in alerting them to dangers. Disarmament is a separate, critically important issue which requires a profound, thorough and scientifically daring approach. I realize that more detailed treatment is needed, but now I will simply outline a few ideas. I consider disarmament necessary and possible only on the basis of strategic parity. Additional agreements covering all kinds of weapons of mass destruction are needed. After strategic parity in conventional arms has been achieved, a parity which takes account of all the political, psychological and geographical factors involved, and if totalitarian expansion is brought to an end, then agreements should be reached first prohibiting the use of nuclear weapons, and later, banning such weapons.

Defence of human rights

Another subject which is closely connected to questions of peace, trust and understanding among countries is the international defence of human rights. Freedom of opinion, freedom to exchange information and freedom of movement are necessary for true accountability of the authorities which in turn prevents abuses of power in domestic and international matters. I believe that such accountability would make impossible tragic mistakes like the Soviet invasion of Afghanistan and would inhibit manifestations of an expansionist foreign policy and acts of internal repression.

The unrestricted sale of newspapers, magazines and books published abroad would be a major step towards effective

freedom of information in totalitarian countries. Perhaps even more significant would be the abolition of censorship which should concern first of all the scientists and intelligentsia of totalitarian countries. It is important to demand a halt to jamming of foreign broadcasts which deprives millions of access to the uncensored information needed to form an independent judgment of events. (Jamming was resumed in the USSR in August 1980 after a seven-year interval.)

I am convinced that support of Amnesty International's call for a general, worldwide amnesty for prisoners of conscience is of special importance. The political amnesties proclaimed by a number of countries in recent years have helped to improve the atmosphere. An amnesty for prisoners of conscience in the USSR, in Eastern Europe, and in all other countries where political prisoners or prisoners of conscience are detained would not only be of major humanitarian significance but could also enhance international confidence and security.

The worldwide character of the scientific community assumes particular importance when dealing with such problems. By its international defence of persecuted scientists and of all persons whose rights have been violated, the scientific community confirms its international mandate which is so essential for successful scientific work and for service to society.

Western scientists are familiar with the names of many Soviet colleagues who have been subjected to unlawful repressions. (I shall confine my discussion to the Soviet Union since I am better informed about it, but serious human rights violations occur in other countries including Eastern European countries.) The individuals I mention have neither advocated nor used violence since they consider publicity the only acceptable, effective and non-pernicious way of defending human rights. Thus, they are all prisoners of conscience as defined by Amnesty International. Their stories have much else in common. Their trials were conducted in flagrant violation of statutory procedures and in defiance of elementary common sense. My friend Sergei Kovalev was convicted in 1975 in the absence of the defendant and counsel, that is, with no possibility whatsoever of defence. He was sentenced to seven years in a labour camp and three years of internal exile for anti-Soviet agitation and propaganda allegedly contained in the samizdat news magazine *A Chronicle of Current Events*, but there was no examination of the substance of the charge.

Comparable breaches of law marked the trials of Yury Orlov, the founder of the Moscow Helsinki Group, and of other members of the Helsinki Groups and associated committees: Victor Nekipelov, Leonard Ternovsky, Mykola Rudenko, Alexander Podrabinek (and his brother Kirill), Gleb Yakunin, Vladimir Slepak, Malva Landa, Robert Nazarian, Eduard

Arutyunian, Vyacheslav Bakhmin, Oles Berdnik, Oksana Meshko, Mykola Matusevich and his wife, and Miroslav Marinovich. Tatiana Osipova, Irina Grivnina and Felix Serebrov have been imprisoned pending trial. Yury Orlov's lawyer missed part of the trial proceedings when he was locked up forcibly in chambers adjoining the courtroom. Orlov's wife was frisked in a crude way and her clothing ripped during a search for written notes or a tape recorder, all from fear that the court's grotesque secrets might be revealed.

Mistreatment

In the labour camps, prisoners of conscience suffer cruel treatment: arbitrary confinement in punishment cells; torture by cold and hunger; infrequent family visits subject to capricious cancellation; and similar restrictions on correspondence.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

E. Yantsevich

Andrei Sakharov at Gorky

They share all the rigours of the Soviet penal regimen for common criminals while suffering the added strain of pressure to "embark on the path of reform", that is, to renounce their beliefs. I would like to remind you that not once has any international organization, such as the Red Cross or a lawyers' association, been able to visit Soviet labour camps.

Political prisoners are often rearrested, and monstrous sentences imposed. Ornithologist Mart Niklus, poet Vasily Stus, physics teacher Olegsei Tikhy, lawyer Levko Lukyanenko, philologist Viktoras Petkus and Balys Gajauskas have all received sentences of ten years in labour camp and five years internal exile as recidivists. A new trial is expected for Paruir Airikian who is still in labour camp. Within the past few days I have been shocked by the fifth (!) arrest of my friend Anatoly Marchenko, a worker and author of two talented and important books: *My Testimony* and *From Tarusa to Siberia*. Imprisoned religious believers include Rostislav Galetsky, Bishop Nikolai Goretoi, Alexander Ogorodnikov, and Boris Perchatkin. Imprisoned workers include Yury Grimm and Mikhail Kukobaka. Alexei Murzhenko and Yury Fedorov are still imprisoned. I shall name

only a few scientists deprived of their freedom; many others could be added to the list: Anatoly Shcharansky, the young computer scientist now famous around the world; mathematicians Tatiana Velikanova, Alexander Lavut, Alexander Bolonkin and Vazif Meilanov; computer scientist Victor Brailovsky; economist Ida Nudel; engineers Reshat Dzhemilev and Antanas Terleckas; physicists Rolan Kadiyev, Iosif Zisels and Iosif Dyadkin; chemists Valery Abramkin and Juri Kukuk; philologists Igor Ogurtsov and Mustafa Dzhemilev; and Vladimir Balakhonov.

A common violation of human rights, and one which especially affects scientists, is denial of permission to emigrate. The names of many "refusniks" are known to the West.

I was banished without a trial to Gorky more than a year ago and placed under a regimen of almost total isolation. A few days ago the KGB stole my manuscripts and notebooks which contained extracts from scientific books and journals. This is a new attempt to deprive me of any opportunity for intellectual activity, even in my solitude, and to rob me of my memory. For more than three years Elizaveta Alexeyeva, my son's fiancée, has been arbitrarily prevented from leaving the Soviet Union. I have mentioned my own situation because of the absence of any legal basis for the actions taken and because the detention of Elizaveta is undisguised blackmail directed against me. She is a hostage of the state.

Fight repression

I appeal to scientists everywhere to defend those who have been repressed. I believe that in order to protect innocent persons it is permissible and, in many cases, necessary to adopt extraordinary measures such as an interruption of scientific contacts or other types of boycott. I urge the use, as well, of all the possibilities of publicity and of diplomacy. In addressing the Soviet leaders, it is important to take into account that they do not know about — and probably do not want to know about — most letters and appeals directed to them. Therefore, personal interventions by Western officials who meet with their Soviet counterparts have particular significance. Western scientists should use their influence to press for such interventions.

I hope that carefully thought out and organized actions in defence of victims of repression will ease their lot and add strength, authority and energy to the international scientific community.

I have entitled this letter "The Responsibility of Scientists". Tatiana Velikanova, Yury Orlov, Sergei Kovalev and many others have decided this question for themselves by taking the path of active, self-sacrificing struggle for human rights and for an open society. Their sacrifices are enormous, but they are not in vain. These individuals are improving the ethical image of our world.

Many of their colleagues who live in totalitarian countries but who have not found within themselves the strength for such struggle, do try to fulfil honestly their professional responsibilities. It is, in fact, essential to work at one's profession. But has not the time come for those scientists, who often exhibit their perception and nonconformity when with close friends, to demonstrate their sense of responsibility in some fashion which has more social significance, and to take a more public stand, at least on issues such as the defence of their persecuted colleagues and control over the faithful execution of domestic laws and the performance of international obligations? Every true scientist should undoubtedly muster sufficient courage and integrity to resist the temptation and the habit of conformity. Unfortunately, we are familiar with too many counterexamples in the Soviet Union, sometimes using the excuse of protecting one's laboratory or institute (usually just a pretext), sometimes for the sake of one's career, sometimes for the sake of foreign travel (a major lure in a closed country such as ours). And was it not shameful for Yury Orlov's colleagues to expel him secretly from the Armenian Academy of Sciences while other colleagues in the USSR Academy of Sciences shut their eyes to the expulsion and also to his physical condition? (He is close to death.) Many active and passive accomplices in such affairs may themselves someday attract the growing appetite of Moloch. Nothing good can come of this. Better to avert it.

Western scientists face no threat of prison or labour camp for public stands; they cannot be bribed by an offer of foreign travel to forsake such activity. But this in no way diminishes their responsibility. Some Western intellectuals warn against social involvement as a form of politics. But I am not speaking about a struggle for power — it is not politics. It is a struggle to preserve peace and those ethical values which have been developed as our civilization evolved. By their example and by their fate, prisoners of conscience affirm that the defence of justice, the international defence of individual victims of violence, the defence of mankind's lasting interests are the responsibility of every scientist.

Gorky 24 March 1981

P.S. After this letter was written, I received word of the tragic death of Juri Kukuk in a labour camp. Tatiana Osipova was sentenced to five years in a labour camp and five years internal exile on 2 April.

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This essay was written by Andrei Sakharov for delivery to the International Conference in Honor of Andrei Sakharov sponsored by The New York Academy of Sciences, the American Institute of Physics and the American Physical Society held on 1-2 May 1981, at Rockefeller University, New York. (English translation courtesy of Khronika Press.)

CORRESPONDENCE

Creationists persist

SIR — No doubt the opponents of evolution are delighted with the fireworks of disagreement that are now being displayed on this subject by biologists who write to *Nature*. Indeed, the critics of Copernicus took heart 400 years ago because scientists could not agree among themselves. However, it is not likely that the scientific process of argument betokens a swing to creationism.

During the Sacramento trial, 2–6 March, the creationists roared as gently as any sucking dove. They said, in the courtroom, that they were not seeking equal time for the teaching of creationism with evolution. In the corridors, talking to reporters, it was a different story. Their veteran leader, Mrs Nell Segraves, said: "We want 50 per cent of the tax dollar used for education to our point of view. We have a lot to undo. Creation/evolution is only the beginning."

The head of the Institute for Creation Research, San Diego, Henry Morris, PhD (Hydraulic Engineering), does not mince matters when he is writing for an audience of believers. He has said that "It is high time that Christians face the fact that the so-called geologic ages are essentially synonymous with the evolutionary theory of origins. The latter, in turn, is, at its ultimate roots, the anti-God conspiracy of Satan himself." He also says the fractures and scars of the Moon and Mars reflect a fight between good and bad angels.

However, the argument used for persuading school boards and legislatures is that "creation science" is a credible variant of conventional science, and deserves equal time. A creationist textbook has anticipated and countered Ashley Montagu's plea (*Nature* 23 April, p.623) that "perhaps the most remarkable and obvious [example of evolution in process] is the well-known case of industrial melanism in moths". Not so, say the creationists, "The moths are just the same as they were before the Industrial Revolution, and they are not becoming anything different . . . Darwin devoted his life in attempting to make it seem reasonable that all forms of life on Earth evolved from one or a few simple forms; this is a very different matter from birds selecting dark or light moths according to the type they can see more readily on a certain background". This argument is strengthened by recent observations that the black morphs are now disappearing because soot is disappearing from trees in the formerly smoky and unkind Midlands.

Creationism is on the march in Oregon, Florida, and Michigan. Governor Frank White signed the Arkansas Bill on 19 March requiring the "balanced treatment" of "creation-science" and evolution in public schools, and admitted afterwards that he had not read all of it. However, on 29 March he told a church congregation that creation-science "is a theory supported by documentation, and evolution-science is the same".

The "equal time for nonsense" position is not one that favours science; science is governed by merit, not fairness. As pointed out by Weber, the position that has the best evidence, has withstood prolonged criticism, and has the mass support from knowledgeable workers in the field, is the position that should

be given the emphasis in education. In science, one theory is *not* just as good as another.

THOMAS H. JUKES

University of California,
Berkeley, USA

Theory upon theory

SIR — The author of the article "How true is the theory of evolution" (*Nature* 12 March, p.75) seemed to misapprehend Popper's refutation principle. He referred not only in the title to the "truth" of a theory but in the article also, where he compounded the error by writing about "proving" theories. Truth and Proof are precisely what Popper's refutation principle does not establish.

It is also unnecessarily narrow to apply Popper's demarcation principle as the exclusive arbitrator in a dispute over the scientific content of a theory. There is no known test or property that demonstrates unequivocally that a theory is scientific or otherwise (see, for example, *The Structure of Scientific Theory*, ed. F. Suppe, 1977). Popper's demarcation principle is not exceptional in this respect; it is probably an analysis most suitably used to explore the scientific content of the theories of physics rather than evolutionary theories, the latter have a historical component the former lack.

Historical theory is often formally different from scientific theory as defined by Popper, as the former can sometimes be confirmed by a finite number of observations. They are confirmed or refuted when all possible observations have been made. In this type of theory, refutation has no logical priority over confirmation — the state of affairs Popper claims for the theories he labels as scientific.

An example of the confirmation/refutation symmetry in historical theory may be found in the theory of evolution, where the gaps in the fossil record are explained sometimes as undiscovered transitional forms. This is theoretically a confirmable historical theory, though in practice the examination of every piece of the Earth's sedimentary rock would be an impossible task. In reality, this theory becomes irrefutable and therefore not scientific according to Popper, unless we interpret his "falsifiable in principle" to mean theoretically testable even if not testable in practice. Popper seems to interpret it thus, as in the *New Scientist* (21 August 1980) he claims historical theories are scientific, but this seems to upset his claim for the priority of refutability over confirmation as a property of scientific theories. On the other hand, should the interpretation be that the explanation of the gaps as transitional forms was in practice irrefutable, it is difficult to accept that finding transitional forms that fit the gaps is in no way a scientific activity.

I would also like to draw to the attention of the author of the article in *Nature* a conclusion of Feyerabend, for example, who states that "relativity theory does not modify Newtonian mechanics, it displaces the theory altogether". The displacement of one major theory by another, according to him, occurs because the two theories represent mutually exclusive universes of discourse and therefore do not have a common language. In this instance, because the two theories embrace incompatible concepts of motion, Newtonian mechanics and

the theory of relativity therefore on Feyerabend's analysis do not have an analogous relationship to Lamarckism and neo-Darwinism. The author's conclusion that Lamarckism would modify neo-Darwinism was correct, but the analogy chosen was probably inappropriate. **BARRIE PEARSON**
Winfrith, Dorchester, UK

Use the library

SIR — The recent letter from Andrew Brooks¹ indicates problems which are encountered by everyone engaged in research. As a Librarian/Information Officer in a small research institute, I often find that the information aspect of research work is approached the wrong way round. The laboratory work is done and a paper almost written before the library is consulted for relevant references. Senior research staff can be particularly guilty, relying strongly on the "old boy" network.

The information explosion of the past 15 years has greatly complicated the task of obtaining relevant information and sophisticated systems have been developed to make the job easier. *Chemical Abstracts* is perhaps the best known information retrieval tool in the sciences. This includes about half a million abstracts per year and anyone not knowing how to use it will have difficulty in retrieving any relevant information. Computerized data bases abound and a directory has been published². *Chemical Abstracts* can also be searched on-line³.

Training in how to obtain information from published sources is surely now essential. I feel it is time for a compulsory, assessed course in information retrieval for undergraduates to be introduced along with other core subject courses.

GILL CROFT

International Tin Research Institute,
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1. Brooks, A. *Nature* 291, 7 (1981).
2. Hall, J.L. *On-line Bibliographic Data Bases* (Aslib, London, 1981).
3. Barker, F.H. *Aslib Proc.* 31, 538 (1979).

SIR — Several months to carry out a literature search! Andrew Brooks' letter (*Nature* 7 May, p.7) amazes me and seems to reveal a lack of initiative by him and by his library. It is part of the job of an academic librarian to explain information handling techniques, whether informally or via a specialist course. In addition, most of us have access to computerized information sources and use them routinely to undertake literature searching for teaching and research staff. Surely a first task for any new researcher (or, indeed, lecturer) is to find out what services the library offers, just as he will investigate available computing facilities. Did Mr Brooks not do this? Conversely, though there are obviously difficulties in institutions with large numbers of researchers, librarians should seek out and make contact with new staff to explain their services. Nevertheless, the SRC might still usefully insist on a course of research methods, including techniques of information retrieval, as an initial part of any PhD programme. **I.R. WINSHIP**

Polytechnic Library,
Newcastle-upon-Tyne, UK

NEWS AND VIEWS

Genes of lymphocytes II: T-cell receptors, idiotypes and the MHC

from Miranda Robertson

OF the inexhaustible enigmas of the immune system, the focal one is certainly the T-cell receptor. Much recent work on the DNA of cloned T cells has been inspired by the hope that T cells, like B cells, recognize antigen by means of binding sites encoded by immunoglobulin variable-region genes. It is well established that T cells do not bear surface immunoglobulin, however, and the evidence that they express V genes is indirect. It is also central to the principal unifying theory of immune regulation.

The theory, originally formulated by Jerne for B cells alone¹, is based on the notion of an interacting network of antibodies which recognize antigenic determinants, known as idiotypes, on the antigen-binding sites of other antibodies. Binding to the idiootype by an anti-idiootype antibody may either induce or suppress the proliferation of the cell bearing it. But much of the immune response and its regulation depend on the activities of T cells: cytotoxic T cells, which kill infected cells; helper T cells, which induce the proliferation of cytotoxic T cells and of some B cells; and suppressor T cells, which suppress all these responses, probably in most cases by acting on the T helper cells that promote them.

One of the most important discoveries of the past decade, therefore, was the demonstration that T-cell help in antibody production could be induced or inhibited by an injection of anti-idiootype antibody². This suggested that T cells bear the same idiotypes, and thus by implication the same antigen-binding sites, as B cells; and made it possible to see how they could participate in the idiootype-anti-idiootype regulatory network proposed by Jerne.

The T-cell receptor

The idea of a T-cell regulatory network based on idiotypes has been enthusiastically embraced by most cellular immunologists, although not all attempts to control immune responses with anti-idiootypic antisera have been uniformly successful. In the hope of clarifying both the cellular interactions and the molecular

signals underlying such regulation, several laboratories have isolated antigen-specific T suppressor and T helper cells which carry idiootype-bearing factors on their surfaces. These factors are shed into the medium of T cells *in vitro*, or extracted from the cells, and can be shown to substitute for them in antigen-specific suppression or help.

The biochemical characterization of T-cell factors has, however, been slow, and is now being rapidly overtaken by the investigation of cloned T-cell lines at the DNA level. The rationale for these investigations is that if T cells are expressing receptors encoded by V-region genes, the V-region DNA of T cells should be rearranged as it is in B cells (see ref. 3 for details of the genes and their rearrangements). The idiotypes expressed by T cells seem to be encoded by heavy-chain V genes of immunoglobulin, so the investigators have used heavy-chain DNA probes to look at T-cell DNA. So far, the results have been negative.

Kurosawa *et al.*⁴, using a J-region probe, have failed to find any functional rearrangement in a cytotoxic T-cell line. This is perhaps not entirely surprising, since cytotoxic T cells do not seem to express immunoglobulin idiotypes (though this in itself is a little unsettling for a network advocate). But at Salt Lake City*, T. Honjo (Osaka University) also reported, on the basis of preliminary data, failure to find rearrangements in T helper-cell lines.

What might these failures mean? One possibility is that the idiotypes expressed on T cells have nothing to do with the V-region genes of immunoglobulins. I. Weissman (Stanford University), playing devil's advocate, pointed out that the T15 idiootype characteristic of the anti-phosphorylcholine antibodies of certain mouse strains is recognized by a monoclonal antibody against Thy-1 antigen — a kind of cross-reaction that has been pointed out before in connection with other cell-surface molecules.

The alternative possibility, which depends on the fact that so far only J-gene

probes have been used, is that in T cells, V_H genes recombine not with J_H genes but with an unknown set of T-cell J genes (J_T genes) elsewhere on the DNA. A report from F. Owen (Tufts University) on a genetically defined, heavy chain-linked T-cell marker mapping downstream of the Ig constant-region genes led to speculation that this might be the site of clusters of the putative J_T and C_T genes.

In any case, one might not expect to find precisely the same genes encoding the antigen-binding regions in T- and B-cell receptors, since the T-cell receptor repertoire is known to be more limited than that of the B cell. One of its most profound limitations is its inability to recognize antigen except when the antigen is associated with a 'self' major histocompatibility complex (MHC) molecule. The mechanism and ontogeny of this so-called MHC restriction are among the most disputed issues of modern immunology, the central questions being whether T cells see both foreign antigen and self-MHC antigen with the same or separate receptors; and whether the receptor repertoire is selected in the thymus — since selected it must be, in order that self MHC be tolerated when free of foreign antigen and attacked when associated with it.

An additional complication for immunologists working on idiootype-bearing T cells and their factors, therefore, is that they must show the appropriate MHC restriction. There are, in fact, several antigen-specific, idiootype-bearing T-cell factors; J. Kapp and her collaborators (Jewish Hospital of St Louis) have recently purified a 25,000-MW suppressor factor specific for GAT and bearing a mouse MHC I-J haplotype, that is particularly interesting because it acts in sub-nanogram quantities *in vivo*.

One of the questions constantly raised about T-cell factors in particular and anti-idiootypic regulation in general is whether they have any physiological relevance. Even the original Eichmann-Rajewsky experiments² are open to criticism on the grounds that they were done with xenogeneic anti-idiootype antisera. In their most recent experiments, Rajewsky and his colleagues used nanogram quantities of syngeneic anti-idiootypic antibodies to

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*The ICN/UCLA Symposium on 'Immunoglobulin Idiotypes and their Expression' was held in Salt Lake City, Utah, on February 8-15, 1981. A report on contributions dealing with immunoglobulin genes has already appeared⁵.

enhance or suppress — depending on timing of administration and dosage of antibody — the responses of mice to hydroxynitrophenol (NP)⁴. Their very latest achievement is to have enhanced the idiotype-positive component of an antibody response to NP by injection of 10 μg of idiotype-bearing antibodies in advance of immunization with NP.

The major histocompatibility complex

If the T-cell receptor remains elusive, the MHC is about to yield up its mysteries to the inexorable advance of the DNA-cloning army. So far, however, the process has only just started, the sequencing has barely begun, and the data largely confirm the existing biochemistry — though there may be some surprises for the geneticists.

MHC antigens, known as HLA antigens in man and H-2 in mouse, are composed of two chains, the smaller of which, β_2 microglobulin, is relatively invariant. The structure of the protein chain shows considerable homology to the constant region of immunoglobulin, but a preliminary analysis of the human genomic DNA (J. Parnes, US National Institutes of Health) suggests that the distribution of introns may be more like that of the variable region.

The larger, α chain, which is highly polymorphic, has only more recently been recognized as having structural similarities to immunoglobulin⁵. Comparing amino acid sequences, J. Strominger (Harvard University) finds 35 per cent homology between the third domain of the α chain of HLA-A and the fourth domain of the immunoglobulin constant region. L. Hood (California Institute of Technology), who has used an HLA probe to identify the H-2 genes, has sequenced the cDNA coding for the third α domain and finds 50 per cent homology with the DNA encoding the fourth constant-region domain of mouse IgM. Moreover, he infers from analysis of the 'wobble' positions of the codons, which have 45 per cent homology instead of the 25 per cent that would be expected by chance, that the homology is due to divergent rather than convergent evolution.

The most startling news came from the California Institute of Technology where, according to E. Moore, densitometric analysis of genomic bands from the H-2 complex of the mouse suggest there may be 15 or so related genes⁶ rather than the four or five predicted by the genetics. Similar data have since been reported by Cami *et al.*⁷, and probably signify the existence of a multi-gene family possibly encoding lymphocyte differentiation antigens.

This raises the question, posed by L. Hood (California Institute of Technology), of how to identify the members of a multi-gene family without knowing what proteins they encode — a problem to which J. Sim (Columbia University) supplied the solution, at least for membrane proteins. She described a system for using cloned DNA to transform

fibroblasts and for assaying the fibroblasts for new surface antigens with cytotoxic lymphocytes, or with antibodies — a technique she and her colleagues have used to identify a genomic clone containing an HLA antigen-encoding sequence.

Nothing in immunology is beyond the wit of man (or woman). T-cell receptor next. □

Blood lead concentrations reconsidered

from Philippe Grandjean

Two recent studies of blood lead concentrations have provided evidence that, compared with the usual average of 10–20 $\mu\text{g dl}^{-1}$ found in Western Europe and North America, levels in remote societies are much lower. Poole and colleagues¹ found a mean blood lead concentration of 5 $\mu\text{g dl}^{-1}$ in 100 children from an unpolluted area in Papua New Guinea and Pionelli and co-workers² established an average blood level of about 3 $\mu\text{g dl}^{-1}$ in 103 Nepalese children and adults from the foothills of the Himalayas. The two investigations were carefully performed in order to avoid the contamination that has marred many lead assays in the past³. The results are supported by an earlier study of Venezuelan Indians⁴ which also indicated low blood lead concentration — below 1 $\mu\text{g dl}^{-1}$ — although the analytical techniques were perhaps subject to some doubt.

Atmospheric lead pollution spreads throughout the world so even population groups living in remote areas may be exposed to lead levels which are higher than natural, pre-industrial levels. One way in which natural levels can be assessed is by the analysis of prehistoric skeletal remains. Such samples, if uncontaminated, can be used to give accurate estimates, since lead accumulates in calcified tissues. Determination of lead in temporal bones and secondary dentine found in the sands of the Sahara has enabled lead levels in Sudanese Nubia to be assessed for the period from BC 3300 to AD 750 (ref.5). The results show that about 5,000 years ago, lead exposure at the Nile banks in Nubia was about one per cent of current exposures in Western Europe and North America. Later, during the Egyptian occupation and with the introduction of metallurgical techniques, tissue lead levels in Nubia increased considerably.

Further evidence comes from Ericson *et al.*⁶ who examined calcified tissues of a

small number of Peruvian mummies. Using sophisticated analyses, they estimated that present-day lead exposures may have increased by about 500-fold above pre-metallurgical levels in Peru.

A report from the US National Academy of Sciences (NAS)⁷ also carefully evaluates the natural occurrence of lead in the environment. Without doubt, lead was never uniformly distributed in the biosphere, and even in pre-industrial times a range of natural exposures would be expected. The NAS committee concludes that current exposure levels in industrialized countries are about 10- to 1,000-fold above natural concentrations.

What is the significance of these figures for human health? Unfortunately, no clear answer is available. Although current background lead levels appear well above those which early man experienced, there is no definite evidence for the threshold at which lead begins to have a toxic effect. Most studies have examined effects at, say, concentrations of lead 1,000–10,000 times the natural level and compared them to a control group with a lead burden 100-fold greater than that. However, toxic effects of lead are now being found at increasingly low levels and the most recent evidence⁸ suggests that they may be close to, or perhaps even below, current background levels.

Until proper studies of low-level lead toxicity, incorporating 'unpolluted' control groups, can be conducted, the recommendation of the NAS committee that "a serious effort should be made to reduce the baseline level of exposure to lead for general population of the United States" should be carefully considered in every industrialized country. □

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Continental crust compromise

from Keith Bell

THE evolution of the continental crust has been a subject of hot debate. Numerous models have been proposed (see, for example, refs 1–4), but most can be roughly placed into one of two groups. Proponents of the first group believe that most continental crust formed fairly early in Earth history, perhaps within the first 500 Myr or so, and that its subsequent reworking gave rise to a variety of granitic products. The alternative proposal argues for a more gradual evolution with additions to the crust being made at intervals throughout its history, either from island arcs at an accreting margin ('lateral accretion') or directly from below ('vertical accretion'). Implicit in some of these models is either an upper mantle that changed continuously or one that had its major differentiation shortly after the formation of the Earth. Also raised in this whole discussion is the origin of granites: are they extracted directly from the mantle? Do they simply represent reworked, older continental crust or are they produced by the mixing of both mantle and crustal components? Recent Sm–Nd work by DePaolo, reported on p.193 of this issue of *Nature*, answers at least some of these questions.

Gast's classic paper⁵ was followed by further attempts to place constraints on the evolution of the crust–mantle system by isotopic means. One obvious approach was to use initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios but this produced surprising results: a large proportion of granitoid rocks were shown to have fairly low and variable initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (<0.706) — a feature which, in its simplest interpretation, favoured a direct mantle origin. Low initial Sr ratios are, however, open to several interpretations including derivation of granites from a source with a similar, low Rb/Sr ratio to that of the mantle (lower crust?); the mixing of continental material with 'primitive' Sr contained either in the mantle, lower crust or within a fluid phase; or derivation from a terrane that somehow had been swept clean of radiogenic Sr (granulite terranes?). Isotopic data from one radiometric system were obviously inadequate to resolve many of the problems of crustal genesis, but the use of initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in conjunction with common Pb data still hinted at a direct mantle origin for most granites⁶.

A new and powerful tool for evaluating the evolution of the upper mantle and the origin of the crust has now been provided by the Sm–Nd method⁷. Because of the somewhat lengthy half life of ^{147}Sm (1.06×10^{11} yr), the ratio $^{143}\text{Nd}/^{144}\text{Nd}$ is usually measured as a fractional difference in parts per 10^4 relative to a reference chondritic reservoir (ϵ_{Nd}). The systematics

of Sm–Nd are very similar to those of the Rb–Sr system but because Sm and Nd are both rare earths, and hence have very similar chemical properties, both parent and daughter nuclides are considered difficult to separate one from another. Current findings suggest that they are not separated by remelting, metamorphism or sedimentary processes⁸. The early realization that the source of most magmas had a Sm–Nd ratio similar to that of chondritic meteorites suggested that the bulk earth had a chondritic composition, a feature supported by other geochemical and geophysical information⁹. As more measurements became available, small but significant differences from the bulk-earth development line were found, particularly the data from ocean-ridge basalts^{10,11}, which helped substantiate the steadily accumulating evidence for a chemically heterogeneous upper mantle. One extremely useful earth model attempts to integrate the isotopic results with what is known about the spatial distribution and volumetric relationships of large-scale basaltic magmatism¹². Although Sm–Nd studies of granitoid rocks are few, the available data support the earlier interpretations of Sr and Pb isotope results from Archaean continental crust. A broad twofold division for all granitoid rocks into those of direct mantle origin and those derived from admixtures of mantle and crustal material is suggested by the isotopic data¹³. Both groups, however, seem to be temporally quite distinct. Granites younger than about 2,000 Myr contain some recycled crustal component, those older are considered primary and of direct mantle origin.

The paper by DePaolo concerns the continental evolution of part of the Proterozoic crust of the southwestern United States and bears, in greater detail, on some of these problems. DePaolo concludes from the Sm–Nd isotopic data that an early event in the Front Range of Colorado at about 1,800 Myr involved the formation of a large segment of continental crust that was derived directly from the mantle, while magmatism during the next 800 Myr, at 1,670, 1,400 and 1,000 Myr ago, involved the formation of granitic bodies from this new crustal segment. (It is interesting to note the very good agreement between the Sm–Nd and Rb–Sr isochron ages.) This story of early mantle material coupled with later crustal involvement agrees rather nicely with the picture emerging from initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios from granitoid plutons found in some orogenic

belts of much younger age (such as the Canadian section of the Appalachian orogen¹⁴). A plot of initial $^{87}\text{Sr}/^{86}\text{Sr}$ versus age shows that the older granitoid bodies have relatively low, mantle-like, initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, while the younger plutons have initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios that are considerably higher (>0.710). The later plutons commonly occur as small, discrete bodies, are commonly peralkaline and sometimes are associated with fluorine, tin and uranium. The overall impression is that mantle-derived material was added to the crust fairly early in the orogenic cycle and that this was followed by substantially smaller amounts of material of quite different geochemical character, which became involved with, or was directly formed from, continental crust.

Of equal interest is the source of the 1,800 Myr material from the southwestern United States because it is relevant to the evolution of the mantle, in addition to placing further constraints on the use of Sm–Nd model ages. The isotopic signature of the older material, quite different from that expected of rocks derived from a chondritic-like source, suggests a depleted upper mantle with a higher Sm–Nd ratio than that of assumed undifferentiated material.

Previous ages from composites of North American continental crust tended to agree with the known geological and geochemical constraints. DePaolo argues, however, that these dates require revision: by using the Sm–Nd data from his depleted upper mantle, he brings them more into line with the known crystallization ages. Although the earlier model ages tend to be underestimated, those from Archaean composites hardly change.

Many problems raised by DePaolo's work are outside the scope of his present paper, which primarily concentrates on the Sm–Nd systematics of the Proterozoic material. One of the more intriguing, however, involves the nature of the early depletion, partly because it brings into focus the meaning of the term 'depleted mantle' and partly because it questions the role taken by this part of the mantle in later crustal generation. Geochemical variations in the mantle can be attributed to a variety of processes including melt extraction, mixing of different mantle zones or metasomatism. The implied widespread nature of DePaolo's depleted mantle suggests that it was formed by an event of planetary proportions. Whatever the nature of the early event (core? crust? formation) it seems to have resulted in a mantle with a higher Sm/Nd ratio than chondrites, which later either generated continental crust directly or became intimately involved with the continental

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crust during its formation. DePaolo's recalculation of crustal-separation model ages certainly implies that large segments of continental crust were derived from a depleted mantle. The question that begs to be asked is 'What are the geochemical implications of an isotopically depleted upper mantle?'. Recent findings, for example, have shown that although initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios from mantle xenoliths in alkalic basalts are variable (with some isotopic ratios indicating a depleted upper mantle), certain major elements, such as CaO and Al_2O_3 , are still present in sufficient amounts to generate basaltic liquids¹⁵. But what of typical continental crust? Can it be derived from a mantle that on the basis of the Sm–Nd data seems to be depleted? A great deal may ultimately depend on the melt volumes extracted from the mantle, spreading rates and the composition of the melts, but the question still remains as to the number of times the same part of the mantle can provide or seemingly provide 'crustal components'.

DePaolo's paper also brings out the feature, already observed from Sr isotopic data, of little or no correlation between initial isotopic composition and rock type. Data from large crustal segments, island-arc volcanics and even ocean-ridge basalts all seem to lie on or close to the depleted, Sm–Nd, mantle development line. This may suggest that most of the chemical characteristics of the various rock types were probably acquired at or close to the time of their formation. In a melting model the chemical composition of the magma would be partly controlled by the minerals present in the mantle and the partitioning of the elements between the liquid and solid phases.

Perhaps additional clues about the evolution of the continental crust can be found elsewhere. In several cases open-system behaviour in the mantle, involving volatiles and CO_2 -rich fluids, has been invoked to explain some of the rare earth

abundances and isotopic data^{16–18}. Several papers have proposed metasomatism in the mantle and terms like 'anomalous mantle' and 'metasomatized mantle' are creeping into the literature. The idea of relating mantle metasomatism to the evolution of the continental crust may seem remote, but the mere facts that it could be considered consistent with some of the isotopic findings and that many granites, such as leucogranites and megacrystic granites, are commonly associated with deep-seated fractures suggest that mantle metasomatism in this context should not be summarily dismissed.

What sort of mantle processes affected which parent–daughter ratios, and the timing of such events, are among the thorny problems that will have to be sorted out. Even in some of the more fertile parts

of the mantle, such as those from which kimberlites are derived, the U–Pb and Rb–Sr ratios are quite different from those of chondrites, suggesting that this part of the mantle may have been affected by a process that left the chondritic Sm–Nd ratio unchanged¹⁹. Much, of course, depends on the nature of the starting material, when it was formed, and the distribution of the chemical heterogeneities within the upper mantle. In spite of these problems there is some indication that the concentrations of primary juvenile gases and their isotopic compositions in mantle-derived materials will help in their resolution. Such information, coupled with the isotopic data from more than one system, may provide further exciting results relevant to the study of the mantle–crust system. □

Clustering of ion channels at the node of Ranvier

from L.C. Fritz and J.P. Brockes

THE segregation or clustering of surface components into discrete and functionally significant domains is an important feature of neuronal cell biology. Obvious examples are the structure of the synapse with its postsynaptic configuration containing a high density of transmitter receptors, and the segregation of various other components to dendrites, cell bodies, axons, or even local domains within these three areas. Axonal calcium channels, for instance, are largely confined to the region of the nerve terminal where they mediate transmitter release. Two systems being used to investigate the mechanisms underlying these processes of segregation are the neuromuscular junction, where acetylcholine receptors are clustered on the postsynaptic muscle fibre, and the node of Ranvier in myelinated axons of the peripheral nervous system. The node is the unensheathed area of axon membrane that lies between two successive myelin segments laid down by Schwann cells. Recent evidence suggests that the nodal membrane contains a high density of certain molecules which mediate electrical excitability and that these molecules are not detectable in the internodal membrane of the myelinated axon.

The passage of the nerve impulse depends on the presence of voltage-sensitive sodium and potassium channels, and it has long been recognized that the nodes are the sites of large inward sodium currents that mediate the saltatory conduction of the action potential¹. More recently, Ritchie and Rogart² have examined the distribution of sodium channels in myelinated frog axons by com-

paring the equilibrium level of binding of the specific ligand tritiated saxitoxin in intact and homogenized axons. Anatomical considerations strongly suggest that the internodal axolemma should not be accessible to ligand in the intact nerve and yet the specific binding did not increase after homogenization. The authors concluded that the sodium channels are present at high density (approximately 10,000 per μm^2 assuming one toxin molecule binds to one channel) in nodes and not detectable (<25 per μm^2) in internodes. Measurements of channel gating currents have also indicated a high density at the node³.

The localization of potassium channels is uncertain. Using voltage-clamp techniques, Chiu and Ritchie⁴ have observed that significant outward current, which is blocked by tetraethylammonium ion, can be measured only after osmotic shock or collagenase digestion which presumably loosens the association of the myelin sheath with the axon. This suggests an internodal or paranodal localization of the potassium channels. In contrast, Binah and Palti⁵ have claimed to detect significant potassium currents at the normal node when particular attention is paid to the holding voltage. This important issue clearly requires further investigation.

There have been several reports of morphological correlates of nodal specialization. In freeze-fracture studies, the nodal membrane has been observed to

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contain a high concentration of large intramembranous particles when compared with the internodal axolemma⁶. Furthermore, Waxman and his colleagues have shown that excitable nodes are specifically stained on the cytoplasmic surface of the membrane by a ferric ion ferrocyanide reagent (FeFCN)⁷. The suggestion that the particle distributions and staining properties of nodes indicate the presence of a high density of sodium channels has not yet been confirmed; these characteristics could relate directly to the channels or to other cytochemical features of excitable nodes.

One particularly interesting question is what role, if any, does neurone-Schwann cell interaction have in the formation of the nodal distribution of sodium channels⁸? The evidence is not decisive, but there are already some intriguing observations. Particle patches observed in freeze fracture, as well as deposition of the FeFCN stain, can be seen in developing axons before the elaboration of the myelin sheath^{9,10}; the patches have also been seen in myelinated regions of the spinal roots in the dystrophic mouse mutant^{11,12}. These observations might suggest that these specializations exist in the axonal membrane before interaction with the Schwann cell. However, the Schwann cells were certainly in contact with axons in the developmental studies even if myelin had not yet been produced. On the other hand, the FeFCN stain has not been observed in myelinated dystrophic axons¹³, and it can be observed over long lengths of demyelinated axons¹⁴. These results might suggest that continuing axon-glial interaction is required for both the generation and maintenance of normal nodal specialization.

Nodal specialization and, in particular, the control of sodium channel clustering are not only interesting but have clinical significance in the recovery of conduction after demyelinating disease, a subject which we have not considered here. Further progress will probably be made with the development of specific cytochemical markers for sodium channels, and the exploitation of defined *in vitro* systems for resolving the nature of neurone-glial interaction. □

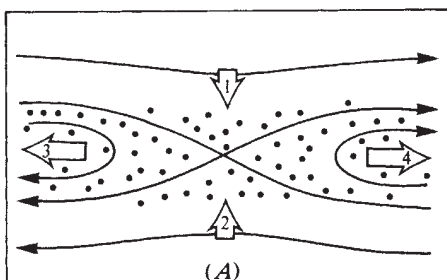
A new magnetic reconnection experiment in the laboratory

from Stanley W.H. Cowley

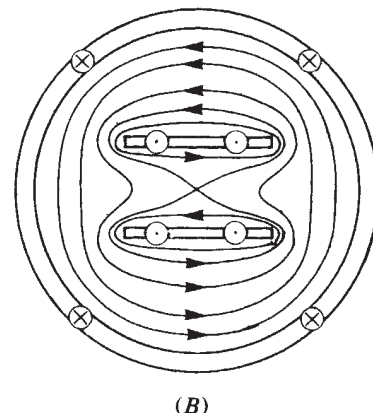
GREAT importance is attached to the magnetic reconnection process in plasma physics. To appreciate why this is so, it is necessary to consider the consequences of a common assumption made in theoretical plasma work, that of perfect electrical conductivity. In a medium with zero resistivity magnetic fields behave in a special way: the magnetic field lines can be considered as being 'frozen' into the medium and can be pictured as being convected along by the fluid motion. Thus, for example, the outer atmosphere of the Sun, which expands outwards at speeds $\sim 400 \text{ km s}^{-1}$, also carries the solar magnetic field into the Solar System because this 'solar wind' gas is a highly conducting fully ionized hydrogen plasma.

An important consequence of the perfect conductivity approximation is that plasmas and fields from separate sources can never mix. When two such plasmas and their embedded fields meet, a thin boundary layer is formed between them which carries the current required by the field jump. No field lines can link across the boundary. However, if finite resistance of the plasma is taken into account, no matter how small, 'reconnection' of the field can occur. This process is sketched in Fig. A, where the dotted region represents the current sheet-separated plasma regions '1' and '2' with antiparallel fields, and the solid arrows give the direction of the plasma flow. The oppositely directed fields move towards each other and 'join' at the centre of the x where the magnetic field strength is zero. The 'reconnected' lines which now link the boundary are then carried away in regions '3' and '4'. Finite plasma resistivity at the field null is required to accomplish this process, allowing the field lines to diffuse through the plasma in this region. Reconnection can therefore only occur when the perfect conductivity approximation is relaxed, although the approximation may be very good elsewhere in the flow, away from the null. Strict antiparallelism of fields '1' and '2' is by no means necessary — fields transverse to the page may be added to the figure and reconnection may still occur under very general conditions.

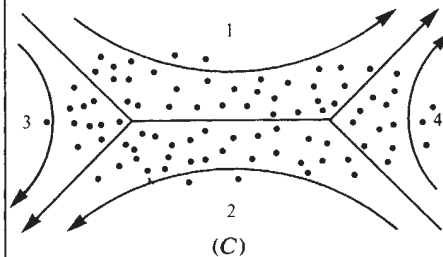
Reconnection is important for two main reasons. First, it changes the topology of the magnetic field and creates direct flux linkages between separated regions, along which plasmas can mix rapidly. Second, energy is liberated from the magnetic field to the plasma in the process, as indicated by the contraction of the field lines in the flow in Fig. A. As a consequence, the plasma in regions '3' and '4' will in general exit as a hot jet. Both aspects are of vital impor-



Sketch of the reconnection process; the lines are magnetic field lines and solid arrows indicate the direction of plasma flow. The dotted region indicates the region of large current density.



Cross-section through the cylindrical experiment vessel, showing the internal plates along which parallel currents are driven, returning along the vessel wall as shown. Circled dots indicate current out of the page, circled crosses current into the page.



Sketch of the observed field configuration at the centre of the Stenzel and Gekelman reconnection experiment during periods of imposed field increase. A neutral sheet configuration is formed rather than an x as shown in A.

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tance, for example, in the Earth's magnetosphere, where reconnection between the Earth's field and the solar wind field results in the magnetic field in the Earth's polar regions being permanently connected to the Sun. On the Sun itself explosive reconnection is believed to be responsible for solar flares, while in the laboratory an apparently similar process occurs in Tokamak 'fusion' machines and results in a disruptive instability in the plasma.

Despite considerable research, the detailed characteristics of the reconnection process have remained elusive. The problem is theoretically difficult because of the requirement to treat the region surrounding the field null, where the approximations usually used in plasma physics are invalid. This is being overcome by computer simulations, but these are, as yet, relatively crude. On the experimental front, extremely detailed plasma and field observations have been obtained from spacecraft in the Earth's magnetosphere, and although ample evidence for an 'open' magnetosphere and particle acceleration has been obtained, it has proved very difficult to pin down the details of the large-scale and temporally varying reconnection processes from single-satellite data. A final approach is to model reconnection in laboratory plasma devices. This has the advantage of repeatability and parameter control, but the detailed diagnosis of the transient plasmas which are produced has been difficult.

Recently, the first results of a new laboratory reconnection experiment have been published (Stenzel and Gekelman *J. geophys. Res.* **86**; 649, 1981; *J. geophys. Res.* **86**; 569, 1981; Wild *et al. Phys. Rev. Lett.* **46**; 339, 1981) in which the system is sufficiently reproducible and the plasma sufficiently accessible to small internal probes that very detailed diagnostics and analysis have become possible. The experimental arrangement is shown in Fig. B. A steady and highly spatially uniform argon plasma is first created inside a cylindrical vessel, which is 2 m long and 1.5 m in diameter, by a pulsed hot-cathode discharge. The plasma density is $\sim 10^{18}$ particles m^{-3} , with electron and ion temperatures of 5 and 0.5 eV respectively, and it is confined radially by a 2×10^{-3} T (20 G) axial magnetic field produced by external coils. A large current is then passed down two internal parallel aluminium plates, returning along the metal wall of the vessel, by discharging a capacitor bank. The current profile so produced resembles that of a highly damped LC circuit; it reaches a peak of ~ 25 kA after ~ 50 μs , after which it decays and, in fact, reverses after ~ 200 μs . At any given time the magnetic field which would be produced in the absence of plasma is indicated in Fig. B. Typically, the field strength is $\sim 10^{-2}$ T (~ 100 G), but is much weaker between the plates, where an x-type null occurs.

During each experiment or 'shot', field

and plasma measurements are obtained at fixed positions and are recorded digitally. For each probe position up to ten shots are recorded and averaged on-line to smooth out fluctuations. The probes are then moved to new positions and the process repeated, about 300 points being so recorded in each of several transverse planes between the plates. The highly detailed data thus obtained are later analysed on a main-frame computer.

The overall behaviour of the system during the experiment is expected to depend primarily on the conductivity of the plasma, which determines the time required for the imposed magnetic field to diffuse into it. If the latter time is long compared with the characteristic time of the experiment (~ 100 μs), the plasma will behave as a perfect conductor, the field will not substantially penetrate the plasma and no reconnection will occur. Instead the plasma should be squashed down into a small volume at the centre of the chamber, leaving a vacuum around the metal conductors containing the applied field, since the energy density of the latter exceeds the initial plasma pressure by a large factor. Alternatively, if the diffusion time is short compared with the characteristic experiment time, diffusion will be dominant and the field should not differ radically from that occurring in the absence of plasma. If the expected plasma conductivity is calculated from the 'classical' formulae based on particle scattering due to particle-particle collisions, then the diffusion time turns out to be ~ 500 μs . This time is fairly long compared with the experiment time, so we expect the perfect conductivity picture to be valid, at least initially. This turns out not to be the case; instead the applied magnetic field rapidly diffuses into the plasma and reconnects across the central field null which is established. However, the field does indeed differ substantially from the vacuum field as a result of the plasma currents. Near the null, in fact, the field does not resemble an x as illustrated in Fig. A but instead flattens into a magnetic neutral sheet as shown in Fig. C. The electron current layer separating the fields is found to be ~ 10 cm wide, although narrower, ~ 1 cm structures have been observed. Such field structures remain stable for ~ 80 μs , evolving in time in response to the changing applied current. During (and due to) the decay phase of the current, however, the sheet tears up to form a magnetic island, although neutral sheet conditions are resumed after the current has been reversed in sense (at 200 μs) and starts to increase again.

Such behaviour indicates that the plasma conductivity is much lower than expected, although not so low that diffusion is totally dominant. This conclusion is confirmed in detail by the experiment diagnostics, which allow a direct measurement of the conductivity to be made, simply by dividing the measured current density by the measured

electric field. It is found that the real resistivity, although highly inhomogeneous, is on average 26 times the classical value. This reduces the diffusion time to a few tens of microseconds, comparable with the time scale of variation of the applied field.

The process resulting in this 'anomalous' resistivity has yet to be identified and is the subject of continuing experiments. The enhanced electron scattering may be due to large electric field fluctuations in the gas caused by an instability associated with the plasma current. The authors point out that significant, about 20 per cent, electron density fluctuations are observed, and that the known conditions for the excitation of ion-sound turbulence are satisfied in the plasma. However, they also note that the observed resistivity does not necessarily maximize in the regions of maximum current as might have been expected, and this requires explanation.

The final result of importance concerns the electric field and the energy deposited in the plasma by the reconnection process. The total electric field is the sum of induced and potential contributions, the first arising from Faraday's law of induction and being proportional to rate of change of the magnetic field (from which it is calculated), while the second contribution is produced by slight separations of electric charge in the plasma and is measured by Langmuir probes. In this experiment, the latter very nearly cancels the former. The variation of the applied field in the reconnection experiment induces an axial electric field of ~ 100 V m^{-1} . The oppositely directed potential field, however, is found to reduce the total field to ~ 10 V m^{-1} throughout the plasma, a value which is, in fact, close to the estimate obtained with Alfvén's (1968) simple neutral sheet model. This experimental result may be of significance in magnetospheric physics, where the role of induced electric fields in particle acceleration has recently been discussed. It also resolves an apparent discrepancy of earlier experiments concerning the energy deposited in the plasma from the field. The rate at which energy is released from the field to the plasma per unit volume is simply given by the product of the current density and the total electric field. If only the induced electric field is considered, as in previous work of this nature, then the energy input estimate will be about a factor of 10 too large due to the partial cancellation effect. In this experiment the energy actually goes largely into electron heating rather than into plasma bulk flows. As a result the electron temperature rises from an initial ~ 5 eV to ~ 15 eV, the peak heating rates and temperature increases occurring at the edges of the neutral sheet rather than at its centre. Electron temperature increases are, in fact, limited to ~ 15 eV by extensive ionization of residual neutral atoms in the vessel during the early phases of the experiment ($\lesssim 30$ μs), and by the excitation of line radiation at later times. $\square$

ARTICLES

Neodymium isotopes in the Colorado Front Range and crust–mantle evolution in the Proterozoic

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Initial $^{143}\text{Nd}/^{144}\text{Nd}$ determined for major rock types of the basement underlying the Rocky Mountains in Colorado indicate that this segment of continental crust was formed from a homogeneous and previously depleted source material in the upper mantle 1,800 Myr ago and contains no identifiable component of older crust. Subsequent magmatic events at 1,670, 1,400 and 1,000 Myr represent mainly intracrustal differentiation with small or no further additions to the crust from the mantle. These results imply that a major increase in the mass of the North American continent occurred over a narrow time interval 1,800 Myr ago. From the isotopic data an empirical model is constructed that may provide accurate crust-formation ages for rocks of all ages. Preliminary results suggest that existing estimates of crustal age distributions may require substantial revision.

UNDERSTANDING how the processes that have shaped and modified the Earth have changed through time is closely tied to the determination of the ages of rocks using radioactive isotopes. The Sm–Nd isotopic method^{1–3} is particularly valuable. Because Sm and Nd have slightly different chemical properties, and because they are relatively immobile during low grade metamorphism, the radioactive decay of ^{147}Sm to ^{143}Nd , and the consequent increases in the ratio $^{143}\text{Nd}/^{144}\text{Nd}$, are reliable indications of when rock material that is now found in the Earth's crust was originally formed by the processes which create crust from the denser, more primitive materials of the mantle^{4,5}. This information, which can be described as the 'crust-formation age' of a rock unit, as opposed to a crystallization age or an age of metamorphism, is crucial for determining if and how the size of the continents has changed through time.

The definition of the Sm–Nd crust-formation age has been based on studies mainly of very old crustal rocks ($\geq 2,500$ Myr)^{2,4–6}. Studies of young rocks have suggested that the model may have serious limitations for younger crust⁷, but data on intermediate-aged rocks have been lacking so that the understanding of the apparent discrepancy between the oldest crust and the youngest crust has been poor. In this study, Sm–Nd isotopic measurements have been made on a previously well-characterized Precambrian basement terrain in the Front Range of Colorado^{8–10}, which has been dated to be 1,000–1,800 Myr old, the intermediate age range where data are lacking. This study was done to test the potential of Sm–Nd crust formation ages as well as to characterize the evolution of this continental province.

Exposures of Proterozoic Precambrian basement are extensive in Colorado in block faulted mountain ranges where Palaeozoic, Cretaceous and Tertiary uplift have exhumed the basement from beneath the Phanerozoic sediment cover (Fig. 1)¹¹. The oldest rocks are gneisses which represent metamorphosed sedimentary and volcanic rocks, known as the Idaho Springs Formation^{8,10}. Recent work has shown that metavolcanic rocks predominate in the northern and southern thirds of the state, whereas metapelitic rocks predominate in the central portion (ref. 12 and C. E. Hedge, personal communication). Depths of erosion have been estimated at 10–20 km; most of the rocks record an upper amphibolite facies metamorphism¹³. Three major intrusive episodes have been identified⁸. The oldest, the Boulder Creek intrusives, were associated with the regional metamorphism. They average granodiorite in composition; Rb–Sr whole rock dating has fixed the age at $\sim 1,670$ Myr. A second intrusive event at 1,400 Myr produced abundant granite with no associated regional metamorphism. The granites are of two-mica type (Silver Plume granite) in the regions where metapelites predominate in the Idaho Springs Formation, and are biotite granites (Sherman–Eolus type) in the regions dominated by metavolcanic rocks (C. E. Hedge, personal communication). The last major Precambrian intrusive event is represented by the Pikes Peak granite (1,015 Myr) which is limited to an outcrop area of $\sim 3,400$ km² at latitude 39°N (Fig. 1). The Proterozoic terrain is bordered on the north by Archaean crust¹⁴ and on the south by younger Proterozoic crust¹⁵. The contact with the Archaean terrain is a shear zone over most of its exposed length which marks a profound lithological discontinuity as well as an age province boundary¹⁶. The Colorado age province extends south-westwards to south-eastern California. It has been separated into two subprovinces

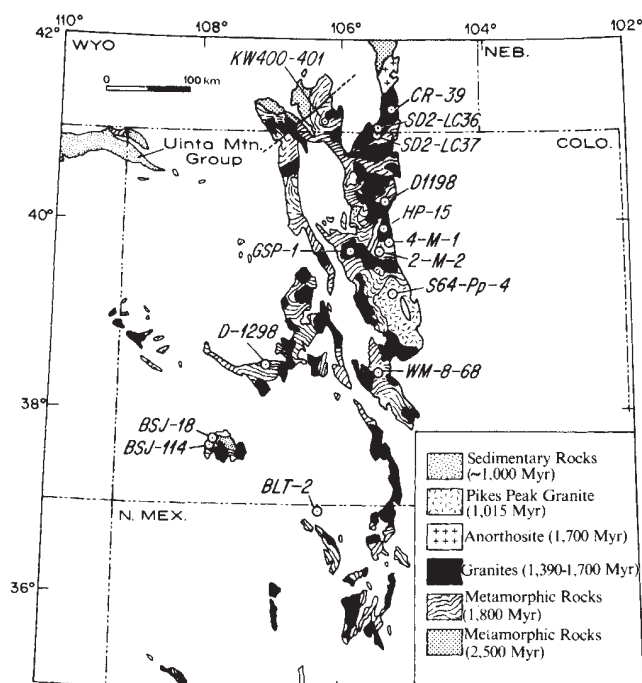


Fig. 1 Generalized basement geological map of the Southern Rocky Mountains, Colorado and New Mexico, USA, showing sample locations (adapted from ref. 11).

of slightly different age on the basis of extensive U–Pb zircon dating^{17,18}. The samples measured in this study are from the northern, slightly older subprovince.

Samples

Sample locations are shown in Fig. 1. All of the samples have been previously measured for Rb–Sr (and in some cases U–Pb) isotopes, and are currently being studied for major and trace element characteristics (ref. 8 and C. E. Hedge, personal communication). The 1,800 Myr metamorphic rocks include two samples of metavolcanic rocks from the Idaho Springs Formation in northern central Colorado and two from the time-correlative Twilight Gneiss in the San Juan Mountains ~300 km to the south-east (Table 1)⁹. Also included in the 1,800 Myr suite are two xenoliths of charnockitic granulite brought to the surface in a kimberlite diatreme near the Colorado–Wyoming border. Four samples of Boulder Creek-type intrusives have been measured, representing a range of chemical types (note Nd concentrations), and widely separated localities. The three samples of Silver Plume granite show strikingly different trace element chemistry (note Sm and Nd), which bracket the range of types found. One sample of typical Pikes Peak granite has been measured. This batholith includes a range of rock types from gabbro to granite, with granite greatly predominant¹⁹. One sample of the Sherman-type granite, from the type locality, has also been measured.

Analytical procedures

The procedures used for Sm and Nd isotopic measurements and the analytical uncertainties have been discussed elsewhere^{20,21}. For this study the precision and accuracy of the determinations of the ¹⁴⁷Sm/¹⁴⁴Nd ratio are particularly important. Tracer solutions of ¹⁵⁰Nd and ¹⁴⁷Sm were calibrated using two ultrapure pieces of Sm and Nd metal (Ames Laboratory, Iowa) as gravimetric standards. Calculated concentrations for the tracers calibrated against these two standards agreed to ±0.05% for ¹⁴⁷Sm and to ±0.2% for ¹⁵⁰Nd. Relative to each other the ¹⁴⁷Sm/¹⁴⁴Nd ratios of the samples are precise to ±0.1%, but a systematic error of up to 0.25% could affect all samples. The effect on the calculated initial ¹⁴³Nd/¹⁴⁴Nd ratios measured have

been normalized to be consistent with data previously reported from Caltech normalized to ¹⁵⁰Nd/¹⁴²Nd = 0.2096 (Table 1). Interlaboratory comparison of Nd isotope ratios shows that ¹⁴³Nd/¹⁴⁴Nd ratios measured at UCLA should be comparable with those measured at Caltech to within ~0.002%²¹, (equivalent to 0.2 ε units as defined below).

The ¹⁴³Nd/¹⁴⁴Nd ratio is also expressed here as ε_{Nd}(*T*), the deviation from the value expected in a chondritic reservoir (CHUR), and is defined as²:

$$\epsilon_{Nd}(T) = 10^4 \left[\frac{{}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{rock}}(T)}{{}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{CHUR}}(T)} - 1 \right]$$

where *T* is time measured backward from the present (age) and

$${}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{CHUR}}(T) = 0.511836 - 0.1967[(\exp \lambda_{\text{Sm}} T) - 1]$$

where λ_{Sm} = 6.54 × 10⁻⁶ Myr⁻¹ (see ref. 22). For a rock of age *T*, ε_{Nd}(*T*) is the 'initial' value of ε_{Nd}; the value at the time of crystallization of the rock, and ε_{Nd}(0) is the measured value.

Results

The results are given in Table 1 and Figs 2 and 3. The gneiss samples define an isochron corresponding to an age of 1,800 ± 90 Myr (2σ) (Fig. 2). These rocks are geographically remote from each other and cannot be cogenetic. There is no *a priori* reason why they should define an isochron as variations in initial ¹⁴³Nd/¹⁴⁴Nd are likely. The data therefore require that the 1,800 Myr age represents a true magmatic age within the age resolution and that the magma source which produced this crust 1,800 Myr ago was homogeneous over distances of several hundred kilometres. Note that the only types of young volcanic rocks which exhibit comparable Nd isotopic uniformity are oceanic island arc rocks²¹. Arcs located far from continental crust show a small range of ε_{Nd} worldwide (Fig. 3), whereas oceanic volcanics in general exhibit a total range from -2 to +13 (ref. 23). The data of Fig. 2 confirm the resistance of Nd isotopes to gross redistribution in crustal metamorphic processes.

The clustering of the initial ε_{Nd} values of the metamorphic rocks is shown in Fig. 3, where initial values for the plutonic rocks are also plotted. The average ε_{Nd} (1,800 Myr) of +3.7 is a

Table 1 Sm–Nd isotopic parameters for Colorado samples

Sample	Description	Sm (p.p.m.)	Nd (p.p.m.)	¹⁴⁷ Sm/ ¹⁴⁴ Nd*	¹⁴³ Nd/ ¹⁴⁴ Nd _m †	ε _{Nd} (0)‡	ε _{Nd} (<i>T</i>)‡
1,800 Myr metamorphic country rocks							
4-M-1	Metarhyodacite (IS)§	7.74	33.86	0.1334	0.511281 ± 22	-10.8	+3.8
2-M-2	Metatholeiite (IS)	3.03	9.96	0.1841	0.511903 ± 26	+1.3	+4.2
					(0.511884 ± 38)	(+0.9)	(+3.8)
BSJ-18	Metadacite (TW)§	4.04	12.06	0.2027	0.512072 ± 28	+4.6	+3.2
BSJ-114	Metatholeiite (TW)	3.62	11.99	0.1826	0.511869 ± 28	+0.6	+3.9
LC-36	Charnokitic granulite	4.07	19.23	0.1281	0.511193 ± 26	-12.6	+3.2
LC-37	Charnokitic granulite	5.29	22.62	0.1413	0.511376 ± 18	-9.0	+3.7
					(0.511364 ± 30)	(-9.2)	(+3.5)
1,670 Myr Boulder Creek intrusives							
BLT-2	Granodiorite	3.06	15.71	0.1180	0.511157 ± 27	-13.3	+3.5
HP-15	Granodiorite	6.06	26.55	0.1379	0.511263 ± 32	-11.2	+1.3
WM8-68	Granodiorite	8.85	42.30	0.1266	0.511154 ± 20	-13.3	+1.7
KW400-401	Granite	4.94	24.03	0.1243	0.511200 ± 23	-12.4	+3.4
					(0.511212 ± 26)	(-12.2)	(+3.6)
1,430 Myr Sherman intrusives							
D1198	Granite	7.72	41.67	0.1221	0.510963 ± 26	-17.1	-1.7
1,415 Myr Silver Plume intrusives							
D-1298	Granite (2 mica)	2.39	7.38	0.1959	0.511837 ± 30	0.0	+0.1
CR-39	Granite (2 mica)	14.24	70.43	0.1223	0.511069 ± 12	-15.0	+0.3
GSP-1	Granite (2 mica)	21.96	172.45	0.0770	0.510605 ± 22	-24.1	-2.5
1,015 Myr Pikes Peak batholith							
S64-Pp4	Granite	18.36	104.62	0.1061	0.511170 ± 24	-13.0	-1.2

* Analytical precision ±0.1%; systematic error due to tracer calibration uncertainties is limited to ±0.25%.

† Measured values, with uncertainty at 95% confidence, normalized to ¹⁴⁶Nd/¹⁴²Nd = 0.63613. Replicate measurement given in parentheses.

‡ Initial values; uncertainties typically ±0.5 ε units.

§ IS, Idaho Springs formation; TW, Twilight Gneiss.

distinctly mantle-type value. Shown schematically is the typical value of ϵ_{Nd} (1,800 Myr) expected in the 2,700 Myr crust which borders the Colorado province to the north²⁴. The ϵ_{Nd} values of 1,800 Myr gneisses give no evidence of influence from older crust. The data require that the Colorado crust be derived from the mantle *in toto* 1,800 Myr ago with the crust formation process involving no reconstitution of older crust.

The line $\epsilon_{\text{Nd}} = 0$ (Fig. 3) has been interpreted as the Nd isotopic evolution of the bulk Earth, or equivalently, of primitive mantle⁴. The initial ϵ_{Nd} of the 1,800 Myr samples demonstrates that this crust was not derived from primitive mantle, but from mantle which had previously acquired an elevated Sm/Nd ratio, a characteristic of parts of the mantle that have been depleted in 'crustal components'. The Colorado crust, therefore, seems to have been derived from depleted mantle, presumably part of the mantle that had been involved in producing crust during the Archaean. Based on data from island arcs²⁵ these conclusions also apply in general to crust being formed from the mantle at present, suggesting that the 'mantle magma source' vector shown in Fig. 3 may be a good approximation to the Nd isotopic evolution of the mantle source of continental crust over the past 2,000 Myr.

In contrast to the gneisses, the Boulder Creek intrusives show a small but distinct range of initial ϵ_{Nd} values ranging from the inferred mantle value near +3.7 down to +1.3. The Silver Plume and Sherman granite samples have ϵ_{Nd} values that are still lower, +0.1 to -2.5, and are far displaced from the mantle ϵ_{Nd} curve (Fig. 3). The Pikes Peak sample also has low ϵ_{Nd} , although it is not lower than those of the Silver Plume samples.

When all of the data on the intrusives are considered, there is a strong correlation between decreasing initial ϵ_{Nd} values and

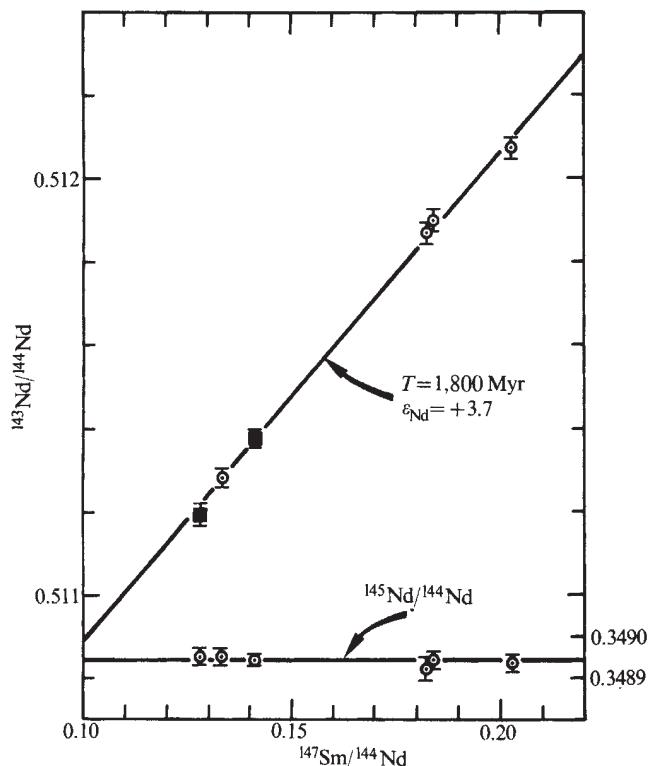


Fig. 2 Sm-Nd isotopic evolution diagram showing data for metamorphic rock samples from the Idaho Springs Formation and the Twilight Gneiss. All six data points lie within experimental uncertainty (95% confidence) of a line whose slope corresponds to an age of $1,800 \pm 90$ Myr. Also shown are the measured $^{145}\text{Nd}/^{144}\text{Nd}$ ratios for the same samples, with the scale shown at the right. This ratio is invariant in natural samples and is measured in this laboratory as an internal check on the measurement techniques for $^{143}\text{Nd}/^{144}\text{Nd}$. The measured values of $^{145}\text{Nd}/^{144}\text{Nd}$ for the six samples lie within error limits of the average value of 0.348945 measured for all samples to date. $\circ$, Metavolcanic; $\blacksquare$, granule xenoliths.

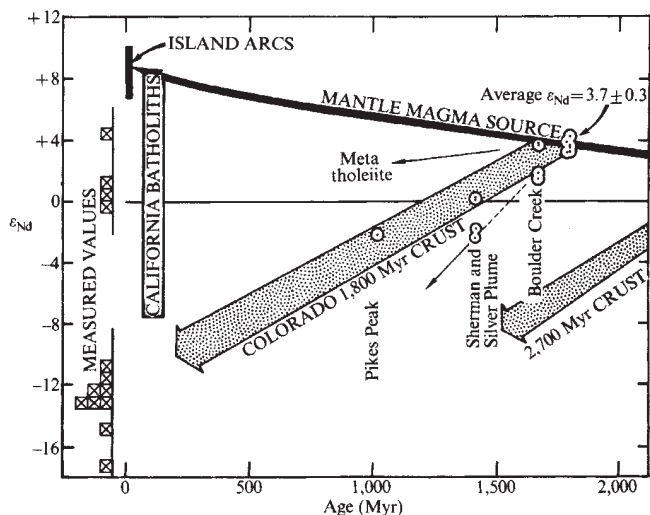


Fig. 3 Measured ϵ_{Nd} values are shown in histogram format at left (GSP-1 not shown). Large arrows show ϵ_{Nd} evolution for typical Colorado crust, and typical 2,700 Myr crust. The curve labelled 'mantle magma source' connects the average ϵ_{Nd} of the 1,800 Myr samples with the average for modern oceanic island arcs. Small arrows give the ϵ_{Nd} evolution for typical 1,800 Myr metatholeiitic basalt and the Silver Plume sample (GSP-1) with lowest initial ϵ_{Nd} . The Colorado crust seems to have been derived solely from the mantle at 1,800 Myr in contrast to the Mesozoic batholiths of California, which are mixtures of mantle-derived components and older crust⁷.

increasing time elapsed since the crust-forming event at 1,800 Myr. Furthermore, the ϵ_{Nd} values fall within or slightly below the approximate average ϵ_{Nd} evolution zone of the 1,800 Myr gneisses (Fig. 3). These observations strongly suggest that the intrusive events at 1,670, 1,400 and 1,000 Myr involved mainly melting of the 1,800 Myr crust rather than derivation of magmas from the mantle. However, two of the Boulder Creek samples have initial ϵ_{Nd} values identical to the inferred mantle value. This could indicate the presence of mantle-derived magma at 1,670 Myr, but would also be consistent with some of the Boulder Creek magmas being formed by melting of the high-Sm/Nd tholeiitic-type 1,800 Myr metabasaltic rocks such as samples 2-M-2 and BSJ-114. For the Silver Plume intrusives, the conclusion based on the isotopic data that they are crustally-derived, is perfectly compatible with their peraluminous bulk chemistry, which independently argues for derivation from the crust. The Sherman intrusives have less aluminous compositions, yet the initial ϵ_{Nd} of the sample measured is identical to the Silver Plume samples. This datum suggests that the Sherman-type granite was also crustally derived; the different bulk chemistry being presumably a reflection of the igneous, rather than sedimentary, character of the crust in northern Colorado.

The interpretation of the Pikes Peak datum is more equivocal, as the alkalic composition of that intrusion represents a fundamentally different magma type¹⁹. It is possible that the Pikes Peak parent magma was derived from a mantle reservoir that had ϵ_{Nd} different from that shown in Fig. 3. However, petrological and other isotopic studies^{19,25} indicate that the Pikes Peak granite represents the end product of a complex magmatic history of crystallization and crustal assimilation, beginning with a mantle-derived basaltic magma. This would leave the granite with a distinctly crustal isotopic composition even though the magmatism originated in the mantle. Further data on the more mafic lithologies of the Pikes Peak intrusion will be necessary to determine the Nd isotopic nature of the source of the parent basalt. The problem of mantle-derived magmas which assimilate copious amounts of continental crust has not been dealt with sufficiently and could also be a factor in the ultimate interpretation of the Silver Plume and Boulder Creek samples.

Conclusions

The present data suggest that the continental crust of Colorado was formed by differentiation from the mantle over a relatively narrow time interval at $1,800 \pm 100$ Myr ago. Subsequently little material was added to the crust from the mantle, with the possible exception of the Pikes Peak event at 1,000 Myr. The production of the Colorado continental crust was apparently not a continuing process, but a fairly sharply defined event. These conclusions, which could apply to the basement of much of the southwestern United States, in conjunction with previous findings⁵, suggest a strong episodicity in the evolution of North America with regard to crust-forming events; crystallization and metamorphic ages show that tectonism and magmatism are more likely to be continuous, or at least to occur more frequently than major crust-forming events. The episodicity in crust production is difficult to understand in the context of current models of plate tectonics, which imply continuous crust production. More data are needed especially from other continents as an individual continent may not preserve a representative history of the crust-forming process.

The possibly well-defined evolution of ϵ_{Nd} in a mantle reservoir which has acted as the source of juvenile crust through the past 2,000 Myr has implications for the dating of crust-forming events and for crust-mantle evolution models. It had been suggested⁴ that crust was derived from mantle reservoirs with $\epsilon_{\text{Nd}} = 0$ and this model was used to calculate⁵ crust-formation ages, T_{CHUR} . However, as shown here, crust is not formed with $\epsilon_{\text{Nd}} = 0$ but from depleted mantle with $\epsilon_{\text{Nd}} > 0$. Hence T_{CHUR} does not give a reliable crust-formation age. Instead, crust-formation ages should be calculated relative to the mantle evolution curve shown in Fig. 3, which can be described by $\epsilon_{\text{Nd}}(T) = 0.25 T^2 - 3T + 8.5$, where T is age. As mantle having this ϵ_{Nd} evolution can be assumed to be depleted with respect to so-called incompatible elements, we denote this revised model age T_{DM} (depleted mantle). As an example of the difference, the T_{CHUR} age of the Colorado samples range from 1,200 to

1,550 Myr, averaging about 1,350 Myr, for the samples with relatively low Sm/Nd. T_{DM} ages are 1,600–1,900 Myr, averaging 1,800 Myr. T_{CHUR} ages underestimate the true crustal age. T_{DM} ages for the Colorado rocks are relatively accurate despite crystallization ages that vary by 800 Myr. This confirms that the fractionated Sm/Nd ratios of crustal rocks relative to the mantle date mostly from the time of original crust formation.

When the crust formation ages of ref. 5 are revised to T_{DM} , calculated ages are plausible and consistent with known crystallization ages. For example, the T_{DM} age of a Grenville composite is 1,300 Myr, identical to the T_{DM} age of a granite from the Llano uplift of Texas, which has been interpreted to be part of the same province. T_{DM} of the North American shale composite becomes 1,750 Myr. Archaean ages change little due to offsetting errors related to revision of the CHUR curve²². T_{DM} ages provide a self-consistent, empirical model for evaluating the formation times of continental crust. This study and previous published Sm–Nd results suggest that earlier estimates of crustal age distributions based on Rb–Sr and K–Ar data²⁶ may have erred by underestimating the amount of old crust. More precise estimates should be possible using T_{DM} when more data become available.

The initial value of ϵ_{Nd} for 1,800 Myr crust could be accommodated for a model of a growing continental crust with no crustal recycling or for a model of a constant-mass crust with decreasing crustal recycling rates towards the present^{27,28}. In either case it is required that the amount of mantle which interacts with the crust be limited to a small fraction (≤ 0.3) of the total mantle. This result supports a two-layered mantle model^{28–30} and suggests that it applies over most of Earth history. The implication is that the basic tectonic–geochemical cycles involving the crust and mantle have not changed from the late Archaean to the present except possibly with respect to rates.

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Biological export of shelf carbon is a sink of the global CO₂ cycle

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Measurements of carbon metabolism, production and exchange along food webs suggest that large fractions of the organic matter produced on continental shelves must be exported to continental slopes. The annual loss of organic matter from continental shelf ecosystems is far greater than in the open ocean. If part of the loss of nearshore primary production has increased in those coastal zones where anthropogenic inorganic nutrient supplies have been consistently increasing since the industrial revolution, then burial and diagenesis of this material in slope depocentres could represent the 'missing BMTs of carbon' in global CO₂ budgets.

THE fate of anthropogenic CO_2 emissions associated with the industrial revolution is not fully known¹. We cannot specify either the steady-state annual fluxes of carbon in a global CO_2 budget or the changes among the major reservoirs of CO_2 (atmosphere, land and ocean) over the past century. At a rate of increase in world fuel consumption of $4.3\% \text{ yr}^{-1}$, a doubling of the CO_2 content of the atmosphere could lead to a 'greenhouse' effect², with a concomitant $2-3^\circ\text{C}$ increase in the ocean's temperature by 2035, similar to the temperature increase since the last glaciation.

The role of the marine biota as a carbon sink has been thought insignificant in the global CO_2 cycle because of nutrient limitation of carbon fixation in the open ocean³. We propose here, however, that a large amount of the CO_2 fixed each year during nutrient-unlimited photosynthesis on the continental shelves is apparently deposited as phytodetrital particles with a C/N weight ratio of $\sim 5/1$ on the adjacent continental slope. After return of nitrogen to the water column during early diagenesis, the refractory carbon particles in the sediment, with a C/N weight ratio of $\sim 10/1$, represent a major organic sink of the global carbon cycle. Part of the nitrogen source for the original coastal phytodetritus might even be manipulated to influence future CO_2 increases of the atmosphere.

Unbalanced global carbon budgets

Of the 5.2×10^9 tons C yr^{-1} now emitted from consumption of fossil fuel and cement production, about half⁴⁻⁶ remains in the carbon pool of the atmosphere (Table 1). The equilibrium between the partial pressure of CO_2 in the atmosphere and that of the upper ocean is such that atmospheric CO_2 usually penetrates the upper mixed layer (~ 75 m) of the sea. During the spring bloom in high latitudes, for example, a primary production rate of only $1-2 \text{ g C m}^{-2} \text{ day}^{-1}$ so far exceeds the atmospheric CO_2 invasion rate that the upper 60 m becomes seasonally depleted of total CO_2 (D. Hood and L. Codispoti, personal communication). The photosynthetic and biogenic CaCO_3 sinks in world-wide surface waters causes the average total inorganic carbon concentration to increase with depth to a maximum at $\sim 1,500$ m, where a CO_2 source occurs within the O_2 minimum layer⁷.

Because of this vertical gradient, it is not clear that a significant fraction of the CO_2 of the upper ocean actually diffuses down across the main thermocline, but surface CO_2 reaches the deeper layers due to bottom water formation at the polar boundaries. The atmospheric CO_2 which penetrates to the deep sea⁸, and that formed by dissolution of the carbonate sediments beneath the main lysocline at ~ 3.7 km is stored as HCO_3^- . In this way $\sim 37\%$ (ref. 3) of the CO_2 emitted from burning fossil fuel, or $\sim 2.0 \times 10^9$ tons C yr^{-1} , may be sequestered as HCO_3^- . Such a polar accumulation of CO_2 , however, is offset to some unknown degree by outgassing of CO_2 at the air-sea interface in the equatorial regions. Without consideration to any other source terms of CO_2 (Table 1) $\sim 0.65 \times 10^9$ tons C yr^{-1} is still 'missing' from such a chemical global budget.

The removal of land biota in undeveloped regions since 1750 may have been a source of CO_2 to the atmosphere. Changing patterns of deforestation mean, however, that the boreal terrestrial ecosystem⁹ may now be a sink of CO_2 , while the tropical forests¹⁰ may be a recent source. How much terrestrial carbon fluxes to the atmosphere or ocean is debatable^{11,12}, but recent estimates suggest a present land source of $1-2 \times 10^9$ tons C yr^{-1} (Table 2). Part of this carbon loss from land could be stored in

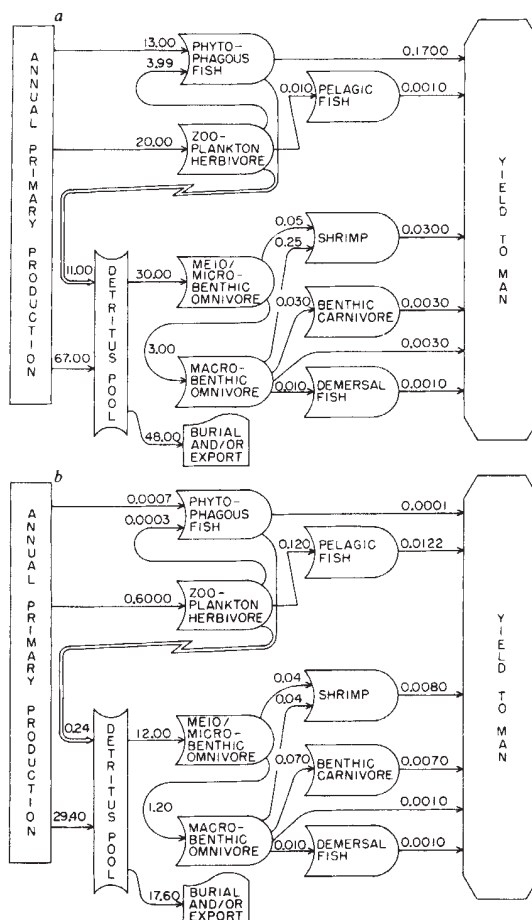


Fig. 1 A carbon budget ($\text{g C m}^{-2} \text{ yr}^{-1}$) of yield to man derived mainly from riverine input of nitrogen on the: a, Louisiana-Texas; and b, West Florida shelves of the Gulf of Mexico.

the intermediate form of both dissolved organic carbon (DOC) and particulate detrital carbon leached into riverine systems. River systems may release as much as 0.8×10^9 tons DOC yr^{-1} to the shelf while sediment accumulation of terrigenous particulate matter¹⁴ within estuarine systems has been estimated¹⁵ to be 0.2×10^9 tons C yr^{-1} . If the land is a source of CO_2 , at least 1.55×10^9 tons C yr^{-1} could be 'missing' in a steady-state global carbon budget (Table 2) that includes fossil fuel and land source terms; atmospheric, DOC, estuarine sediment and deep ocean chemical sinks; but ignores the marine biota as a possible sink.

Marine detrital sink

A marine primary production^{11,15} of $\sim 26 \times 10^9$ tons C yr^{-1} can be partitioned into 20×10^9 tons C yr^{-1} derived from the open ocean ($\sim 3 \times 10^8 \text{ km}^2$) and 6×10^9 tons C yr^{-1} from the continental shelves ($\sim 3 \times 10^7 \text{ km}^2$). Over 90% of the photosynthetic uptake of CO_2 in the open ocean is grazed within the upper 100 m, however, with most of the phytoplankton carbon and nitrogen recycled by herbivores as CO_2 from respiration and as ammonium from excretion. ^{15}N estimates of the daily nitrogen demand of oceanic primary production suggest¹⁶ that at least 90% is met by the recycled NH_4^+ . Those organic detrital particles, which are not grazed and sink out of the euphotic zone (~ 100 m) of the open ocean, amount to $\sim 2 \times 10^9$ tons C yr^{-1} metabolized at depths down to 1,000–2,000 m, where the CO_2 produced is sequestered within the bicarbonate cycle. Heterotrophic metabolism within the O_2 minimum layer poises the diffusion gradient of total CO_2 towards the surface, that is the diffusive flux of CO_2 should be towards the euphotic zone⁷. Because CO_2 returns to the euphotic zone with the 'new' nitrate

Table 1 An unbalanced chemical budget of the global CO_2 cycle ($\times 10^9$ tons C yr^{-1})

Sources	Fossil fuel emission	5.20
	Equatorial outgassing	?
	Volcanism	0.05
Sinks	Carbonate neutralization	2.00
	Atmospheric storage	2.60
Missing carbon		0.65

diffusing upwards to support primary production, the importance of open ocean organic detritus as a net sink for CO_2 is probably small¹⁶. Of the minute amount ($\sim 1\%$ of the open production) of particles that reach 4,000–5,000 m, a fraction of this organic matter with a C/N ratio ≥ 10 is finally incorporated (Table 2) in bottom sediments of a similar C/N ratio¹⁷.

Phytoplankton populations of the continental shelf, in contrast to those of the open ocean, do not seem to be either nitrogen-limited or grazer-controlled for parts of the year but rather light-limited as evidenced by spatial and temporal outbreaks of algal blooms with C/N ratios ≈ 5 in coastal waters^{14,18,19}. In further contrast to the oligotrophic open ocean, ^{15}N estimates of nitrogen uptake by coastal phytoplankton suggest that at least 50% of the daily nitrogen demand of photosynthesis is met in the form of nitrate off Peru, western Mexico²⁰, California²¹, in the Middle Atlantic Bight²², in the antarctic²³, and in the south-east Bering Sea²⁴. The major sources of nitrate in these systems seem to be river runoff²⁵ and cross-shelf exchange of subsurface slope water onto the shelf^{24,26,27}; the importance of nitrification on the shelf is unknown.

After the decline of the Peruvian anchoveta fishery, removal of fish by man from each shelf amounts to $<1\%$ of the annual primary production²⁸. Because nitrate supply is considered an estimate of available 'new' production^{16,29}, because fish yield is not a significant particulate nitrogen loss, and because plankton biomass is not continuously increasing on the continental shelf, $\sim 50\%$ of the 'new' primary production should at times be exported from the shelf water column each year as opposed to the $<10\%$ loss derived from 'new' production of the open ocean euphotic zone¹⁶. Annual carbon budgets for the shelf food web supported by riverine input of nitrogen in the Gulf of Mexico (Fig. 1), from shelf-break input within the Bering Sea (Fig. 2) and the Peru upwelling ecosystem²⁸, and from the more complex sources of nitrogen in the New York Bight³⁰, all indicate that 50% of the primary production is apparently not consumed by shelf organisms at 20, 30 or 60° latitudes.

Table 2 An unbalanced biological budget of the global CO_2 cycle ($\times 10^9$ tons C yr^{-1})

Sources	Fossil fuel emission	5.20
	Equatorial outgassing	?
Sinks	Volcanism	0.05
	Deforestation	2.00
	Atmospheric storage	2.60
	Riverine release of DOC	0.80
	Terrestrial sediment	0.20
	Open ocean detritus	0.10
	Carbonate neutralization	2.00
Missing carbon		1.55

A balanced budget of the carbon requirements of the demersal food web on the Scotian shelf³¹ was obtained using net production/biomass ratios and transfer efficiencies similar to those above. However, this estimated production, $102 \text{ g C m}^{-2} \text{ yr}^{-1}$, did not include the spring bloom which amounts to 40–50% of the yearly production within the adjacent Mid-Atlantic Bight. The similarity between the cross-shelf nitrate flux and fish yield of the Nova Scotian and Bering shelves²⁴ suggests that the annual primary production may be the same. Using an estimate of primary production of at least $200 \text{ g C m}^{-2} \text{ yr}^{-1}$, we find a budget for the Nova Scotian shelf could not account for 50% of the annual production. Furthermore, a phytoplankton cell, falling to the bottom of the Scotian shelf, might be entrained in the subsurface offshore flow near the shelf-break³²; sediment chlorophyll content to 800 m on the upper slope is, in fact, 30 times greater than on either the Scotian shelf or the lower slope³¹.

Offshore flows of water also occur during upwelling off Peru and on estuarine-type shelves such as the New York Bight and the Bering Sea, suggesting that cross-shelf transport of biogenic particles is a ubiquitous phenomenon, perhaps intensified as a result of winter storms. Deposition of organic carbon in shelf sediments has been suggested^{15,31} as a sink in the global carbon

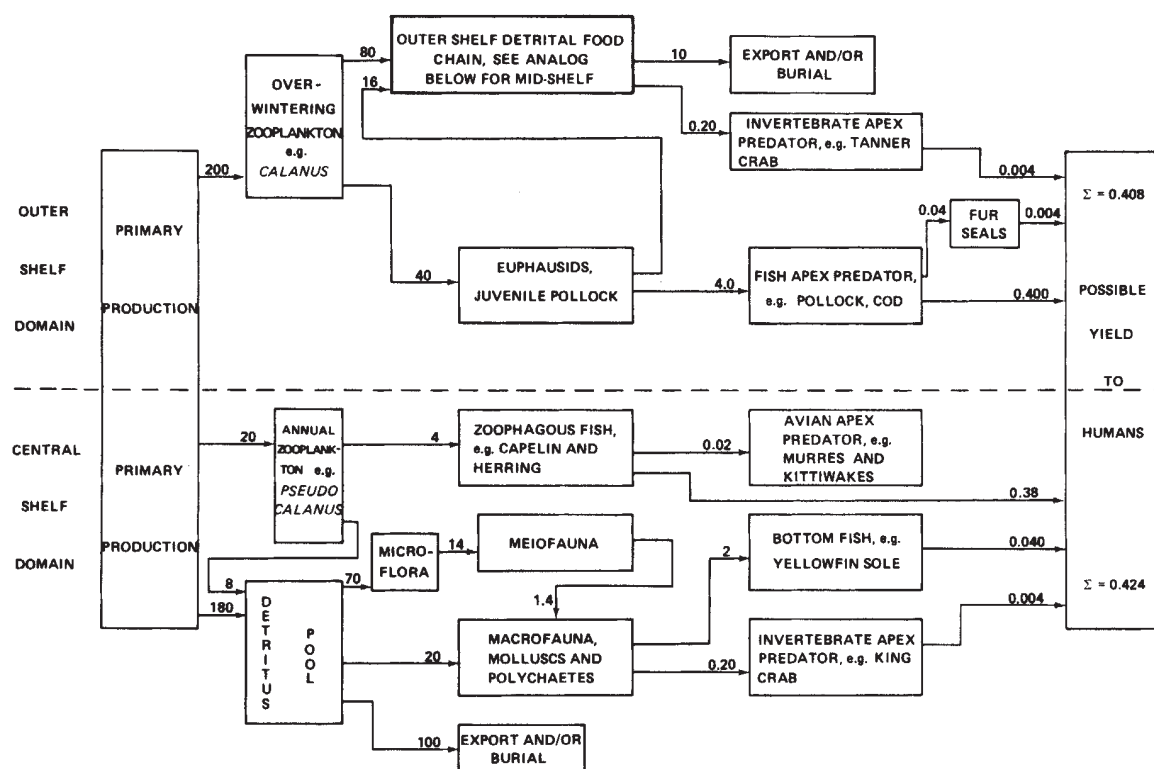


Fig. 2 A carbon budget ($\text{g C m}^{-2} \text{ yr}^{-1}$) of yield to man derived mainly from the shelf-break input of nitrogen within the outer and central shelf domains of the Bering Sea.

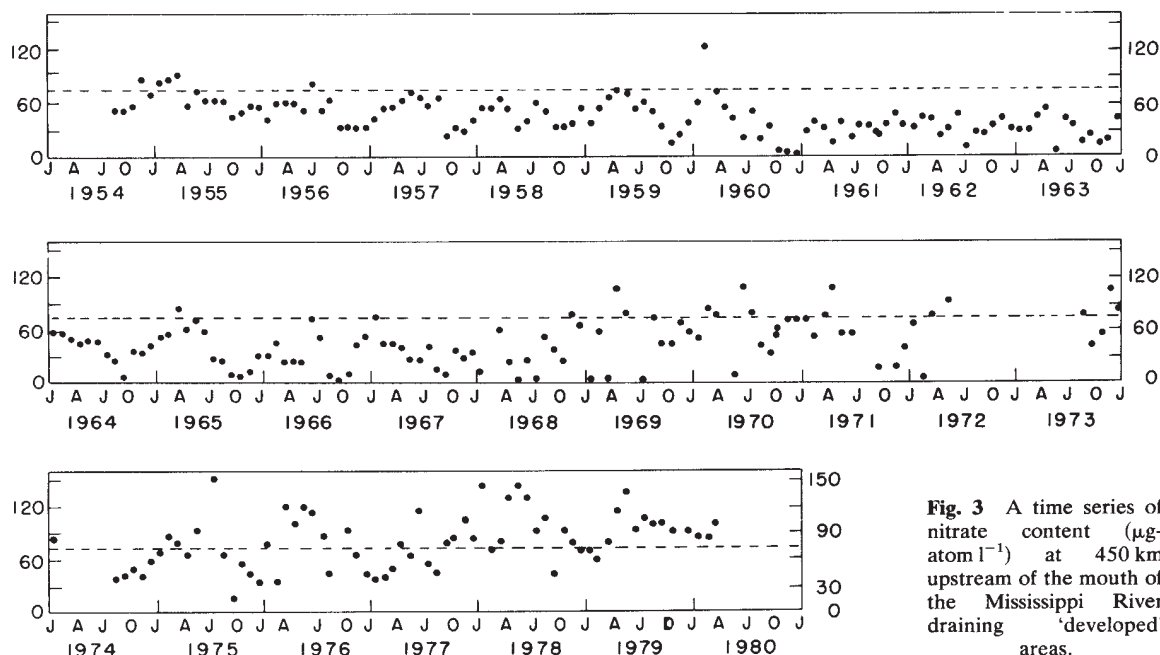


Fig. 3 A time series of nitrate content ($\mu\text{g-atom l}^{-1}$) at 450 km upstream of the mouth of the Mississippi River draining 'developed' areas.

cycle, but most recent shelves have sediments consisting of relict sands with $<0.5\%$ carbon content. However, surficial sediments of the upper slopes, for example, off the US east coast and the Gulf of Mexico, contain low C/N ratios (~ 5) with organic carbon concentrations $>1\%$, implying marine origin¹⁴. After early diagenesis, the C/N ratio of particulate matter in these sediments increases to ≥ 10 . That is for every 5 g of carbon and 1 g of nitrogen returned to the slope water column, a maximum of 5 g C could be left behind in the sediments. A carbon export of 3×10^9 tons C yr^{-1} from the total shelf production could now be deposited¹, with perhaps 1.5×10^9 tons C yr^{-1} accumulated¹⁴, within slope depocentres, constituting the sink of missing carbon (Table 2) in the global CO_2 budget. Estimates of the annual detrital nitrogen export from some of these shelves are balanced by independent budgets of shelf nitrogen recycling and of the return flux of nitrate from offshore and riverine sources^{24,28,30}.

Transients in anthropogenic nitrogen loading

Most of the CO_2 produced by burning fossil fuel has been introduced into the atmosphere within the past two decades^{5,6}; before the 1950s most of the emitted CO_2 was thought to have been stored in the ocean³⁴. Presumably a transient in one or more of the sinks of CO_2 (Table 2) must have occurred in response to this increase of the fossil fuel source. However, a decline in temperate forest emission⁹ of CO_2 from a source of $\sim 3 \times 10^9$ tons C yr^{-1} in 1850 to a net sink after 1950 may have been partially balanced by the increase in fossil fuel emission. For example, ignoring both marine photosynthesis as a sink and the land as a source term, a budget³⁵ of chemical storage of CO_2 within the deep ocean from 1850 to 1950 may have over-

estimated³⁶ the initial carbon content of the pre-industrial atmosphere by $\sim 50 \times 10^9$ tons C. In this case, at least 0.5×10^9 tons C yr^{-1} could have been lost to an unknown biotic sink without considering the changes in deforestation during most of this century.

Other analyses of abiotic storage of CO_2 in the ocean by vertical mixing³⁷ and/or polar sinking³⁸ over the past few decades neglect biological fixation of carbon in the sea and cannot account for all the CO_2 recently emitted by fossil fuel burning. The open ocean photosynthetic loss from the water column is unlikely to have increased¹⁶ as a result of either natural or anthropogenic nutrient loading¹⁸. The shelf ecosystems respond to seasonal nutrient enrichment, however, when light limitation ceases, by formation of spring phytoplankton blooms¹⁸, which seem to be exported to the slope depocentres. In summer stratified conditions of nitrogen depletion, anthropogenic nutrient addition to surface coastal waters would also increase the yield or export from this ecosystem. Poor records of primary production in the ocean on the decadal time scale³⁹ mean that it is difficult to demonstrate the eutrophication of coastal waters by direct observation. The daily production of the mid-shelf spring bloom in the New York Bight has apparently not changed, for example, over the past 25 yr²⁷, but spatial sampling was inadequate to determine whether nearshore production had increased. The Baltic⁴⁰ and Waddensea are exceptions, where the data base indicates that annual production of the nearshore zone has apparently increased from 80 to $240 \text{ g C m}^{-2} \text{ yr}^{-1}$ over the past 20 yr⁴¹.

The lack of change in nutrient content at midshelf off New York, away from the influence of the Hudson River over the past 25 yr²⁷, suggests that only coastal anthropogenic sources of nutrients, not shelf-break sources, could have reasonably changed in response to the industrial revolution. The phosphate content⁴² of the Rhine River has, in fact, increased 10-fold over the past 50 yr. The nitrogen content of the Rhine⁴³ almost doubled between 1959 and 1968, while that of the Seine (Y. Monbet, personal communication) increased fourfold to $\sim 400 \mu\text{g-atom N l}^{-1}$ from 1965 to 1975. The mean winter nitrate content of the North Sea shelf is also at least two- to fourfold higher off the Thames estuary, the Wash and the Rhine estuary than at the edge of the shelf⁴⁴. Indeed, as much as $350 \mu\text{g-atom NO}_3 \text{ l}^{-1}$, ~ 10 -fold the concentration of NO_3 in deep slope water, has been found both in the Scheldt estuary and 10 km off the Belgian coast⁴⁵.

Table 3 Major river discharge ($\times 10^3 \text{ m}^3 \text{ s}^{-1}$) and nitrate content ($\text{mg-atom NO}_3 \text{ m}^{-3}$) in relation to human population centres (1970 census)

Oligotrophic N sources (5.5×10^8 people) Africa-South America-Oceania				Eutrophic N sources (30.1×10^8 people) North America-Europe-Asia			
River	Flow	NO_3	Ref.	River	Flow	NO_3	Ref.
Amazon	180	10	53	Yantze	34	70	*
Congo	41	7	54	Mississippi	18	150	†
Orinoco	20	8	*	Mekong	11	75	55
Niger	6	8	54	Rhine	2	350	43

Personal communications: * J. Edmond; † C. Demas.

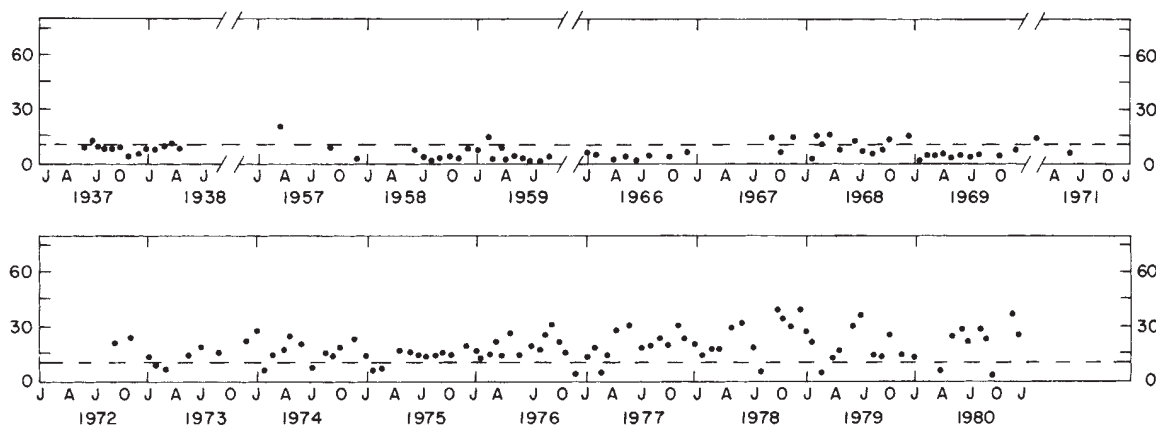


Fig. 4 A time series of the nitrate content ($\mu\text{g-atom l}^{-1}$) at 50–100 km upstream of the mouth of the Altamaha River draining 'undeveloped' areas.

Within North America, the nitrate content of the Mississippi River (Fig. 3) has at least doubled to $\sim 150 \mu\text{g-atom NO}_3 \text{ l}^{-1}$ during spring flood over the past 25 yr, while $30 \mu\text{g-atom NO}_3 \text{ l}^{-1}$ can still be found downstream on the otherwise oligotrophic shelf at its mouth¹⁵. Data^{46–49} for US rivers which drain other heavily populated areas suggest that their nitrogen content has also increased over the past 25–50 yr from sewage inputs, agricultural fertilizers and nitrate release to groundwater after deforestation⁵⁰. Similarly, US rivers which drain areas of low populations used to contain 10-fold less inorganic nitrogen ($\sim 7\text{--}10 \mu\text{g-atom N l}^{-1}$) than the above rivers before 1970, but the nitrate content of the Altamaha (Fig. 4) and Pamlico⁵² rivers is now $\geq 30 \mu\text{g-atom NO}_3 \text{ l}^{-1}$.

The location and discharge of the world's 30 largest rivers further suggests that a similar freshwater flux occurs from 'undeveloped' areas ($\sim 286 \times 10^3 \text{ m}^3 \text{ s}^{-1}$) compared with those from 'developed' areas ($\sim 234 \times 10^3 \text{ m}^3 \text{ s}^{-1}$) with a sixfold larger human population, that is greater anthropogenic nitrogen sources. If we assume that half the world's total river discharge ($\sim 4 \times 10^{16} \text{ l yr}^{-1}$) has not yet been affected by deforestation, sewage and fertilizers in these underdeveloped countries, and that the low nitrate contents of the Amazon⁵³, Congo⁵⁴, Orinoco (J. Edmond, personal communication) and Niger⁵⁴ Rivers ($\leq 10 \mu\text{g-atom NO}_3 \text{ l}^{-1}$) are typical of these regions (Table 3), nitrate input to the shelves from such drainage systems is $\sim 0.3 \times 10^{13} \text{ g NO}_3\text{-N yr}^{-1}$. If we also assume that the other half of the world's river discharge now has at least $60 \mu\text{g-atom NO}_3 \text{ l}^{-1}$ due to population distribution and the industrial revolution ($> 70 \mu\text{g-atom NO}_3 \text{ l}^{-1}$ is found in the Hudson, Potomac, Susquehanna, Delaware, Mekong⁵⁵ and Yantze (J. Edmond, personal communication) rivers and recall concentrations of $150\text{--}300 \mu\text{g-atom NO}_3 \text{ l}^{-1}$ in the Mississippi, Seine, Rhine and Scheldt rivers) the annual nitrate input from these developed terrestrial ecosystems would be $\sim 1.7 \times 10^{13} \text{ g NO}_3\text{-N yr}^{-1}$.

A present input of $2.0 \times 10^{13} \text{ g NO}_3\text{-N yr}^{-1}$ from river runoff would be at least treble that of $0.6 \times 10^{13} \text{ g NO}_3\text{-N yr}^{-1}$ estimated more than 25 yr ago⁵⁶, from data taken more than 50 yr ago⁵⁷. Using a similar unrevised estimate of $0.8 \times 10^{13} \text{ g NO}_3\text{-N yr}^{-1}$ in river runoff, it has been suggested⁵⁸ that only $\sim 10\%$ of the nitrogen fertilizers previously applied to fields had yet to be released from the soil to streams by 1970. In terms of a seasonal range, the inorganic nitrogen content of rural land runoff in Ohio⁵⁹ varies during the year from ~ 10 to $600 \mu\text{g-atomic N l}^{-1}$ as reflected in the monthly nitrate content of the downstream Mississippi River (Fig. 3). The long-term trend of this and the Altamaha (Fig. 4) time series over the past 25 yr, however, suggests that NO_3 concentrations of these rivers has doubled within the past decade. If mobilization of nitrogen from soil pools is now increasing, we would expect future increases of primary production in the coastal zone and greater export of this carbon to the slope.

In fact, other estimates⁶⁰ of riverine nitrogen loading range as high as $3.5 \times 10^{13} \text{ g N yr}^{-1}$ and rivers are not the only source of anthropogenic nitrogen inputs to coastal waters. An analysis⁶¹ of the present annual nitrogen loading from coastal sewage outfalls, waste dumping and river input to the shelves of the New York Bight and the Mediterranean, Seto Inland, Baltic, North and Irish Seas suggest a range of $1\text{--}3 \times 10^4 \text{ tons N yr}^{-1}$ ($10^6 \text{ persons}^{-1}$) for each of these areas. Extrapolating the mean of this estimate to half of the world population of $30 \times 10^8 \text{ persons}$ for just coastal communities of North America–Asia–Europe (Table 3), we obtain an estimate of $3 \times 10^7 \text{ tons N yr}^{-1}$, or $3 \times 10^{13} \text{ g N yr}^{-1}$ from both rivers and other sources of nitrogen within the developed regions.

Conclusions

With a C/N ratio of 5:1 for primary production/nitrogen uptake, this anthropogenic loading of 'new' nitrogen would allow a coastal export of $0.15 \times 10^9 \text{ tons C yr}^{-1}$ without considering the carbon fixed in recycling this nitrogen source. The dissolved nitrogen pool of nearshore shallow waters is recycled ~ 10 times during the year⁶², which is reflected by observations that indicate recycled nitrogen amounts to 90% of the daily ^{15}N uptake of phytoplankton in the Hudson⁶³ and Chesapeake⁶⁴ plumes. These data suggest a primary production of $1.50 \times 10^9 \text{ tons C yr}^{-1}$ from this source, that is, $\sim 25\%$ of the present shelf production. After each change of the particulate C/N ratio from 5/1 to 10/1 during the nearshore remineralization cycle, $0.75 \times 10^9 \text{ tons C}$ of refractory carbon could be available for export each year as a result of this nitrogen loading. Once produced on the outer and inner shelf, organic matter of a C/N ratio of either 5/1 or 10/1 tend to be transported with those fine-grain sediment particles, which, escaping the sediment traps of the estuaries, transit the continental shelf and are deposited on the upper slopes^{65,66}.

Our analyses suggest that up to half of the estimated $1.50 \times 10^9 \text{ tons C yr}^{-1}$ of the slope carbon sink could result from increased eutrophication since the industrial revolution. A combination of outer shelf ungrazed phytodetritus with a C/N of 5/1 and nearshore reworked detritus with a C/N of 10:1, sequestered together on the slope, would constitute both the missing carbon (Tables 1, 2) and the particle flux required as a sink for ^{210}Pb budgets⁶⁷. The biological export of shelf carbon in transient and steady-state budgets of the global CO_2 cycle could present an important management strategy. Increased eutrophication of a well-flushed coastal zone should lead to both increased primary production (fixation of CO_2) and export, not anoxia⁶⁸, such that sewage waste disposal problems might be coupled with averting increases in the CO_2 content of the atmosphere.

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Homology between the primary structure of α -fetoprotein, deduced from a complete cDNA sequence, and serum albumin

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The entire DNA complementary to murine α -fetoprotein mRNA has been cloned in the plasmid vector pBR322 and its complete nucleotide sequence determined. The deduced amino acid sequence identifies a hydrophobic prepeptide of 19 amino acids and the complete primary structure of α -fetoprotein. Its structure reveals extensive homology to serum albumin and suggests that there are 15 disulphide bridges in the α -fetoprotein molecule, all at the same positions as those found in albumin. The AFP molecule can be organized into a three-domain structure almost identical to that of serum albumin, suggesting a common ancestral origin of the two proteins.

α -FETOPROTEIN (AFP), a serum protein with an apparent molecular weight¹ of 72,000, is made and secreted by the fetal liver² and yolk sac³ of all mammals so far studied. The serum concentration of AFP decreases rapidly during fetal development to a residual level in adulthood but rises again in individuals with hepatomas or germ-cell tumours⁴⁻⁶. Elevation of serum AFP levels is also observed in a variety of pathological conditions^{6,7} and in certain human fetal malformations such as neural

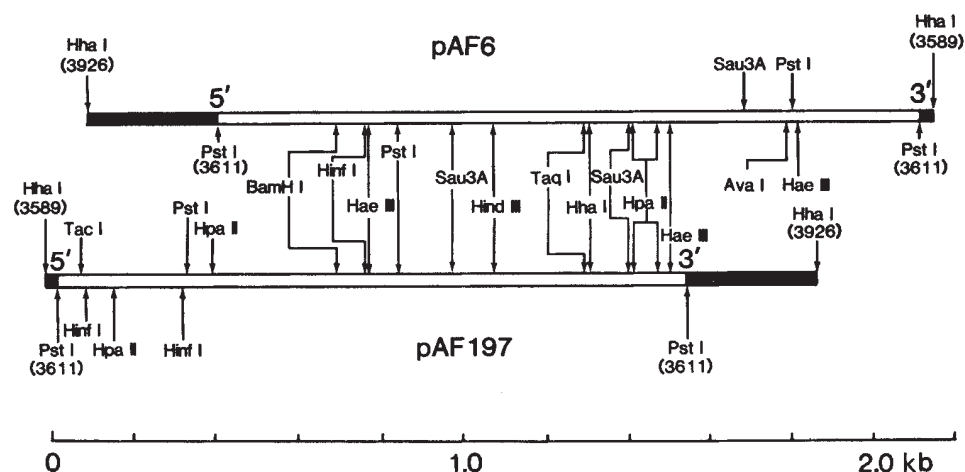
tube defects^{8,9}. The exact function of AFP is unclear, although similar biological and physicochemical properties suggest that it may be the fetal counterpart of adult serum albumin.

Human AFP and albumin have been reported to exhibit some homology in amino acid sequence¹⁰. However, a comparison of the amino-terminal sequence of human, murine and rat AFP with sequences of the respective albumins showed no sequence homology between the two proteins¹¹. Furthermore, cDNA to rat AFP mRNA does not cross-hybridize to rat albumin mRNA in moderately stringent conditions^{12,13}. The determination of a partial cDNA sequence of rat AFP^{14,15} has recently revealed a partial homology between the rat AFP and serum albumin

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Fig. 1. Restriction endonuclease cleavage sites on the overlapping murine AFP cDNA inserts in the recombinant plasmids pAF6 and pAF197. The map shows the inserted AFP DNA (open bar) and its orientation in the pBR322 plasmid DNA (black bar). The murine-derived DNA is inserted at the *Pst*I site of the plasmid DNA as described in detail previously¹⁶. The AFP mRNA sequence is presented by the upper DNA strand of the pAF6 and pAF197 map, with the 5' and 3' ends as indicated. Numbers on restriction sites within the pBR322 sequence, like *Pst*I (3,611), are taken from available sequence data from Sutcliffe³⁵ and represent positions on the sequence map of pBR322, counted from the single *Eco*RI site on the circular DNA molecule. kb, Kilobases.



amino acid sequence. We now describe the cloning and nucleotide sequence determination of the entire DNA complementary to murine AFP mRNA and analyse the deduced primary amino acid structure and disulphide bonding pattern of the AFP molecule in the light of its homology to serum albumin.

The recombinant plasmid pAF6

We have previously reported¹⁶ the construction of a cDNA clone containing about 1.65 kilobases of mouse AFP mRNA sequence (Fig. 1). The clone was identified on the basis of a hybrid-arrested *in vitro* translation of mouse yolk sac mRNA, which specifically arrested the translation of the AFP mRNA species. As shown below, nucleotide sequence analysis has revealed that the cDNA sequence in pAF6 corresponds to the 3' region of the AFP message. This clone does not contain the 5' sequence corresponding to the known 20 amino acids at the amino terminus of mouse AFP. Comparison of the size of the protein¹ and the size of the cDNA in pAF6 indicates that about 400 nucleotides of additional coding sequence are required to complete the 5'-terminal sequence of the AFP message.

Cloning of cDNA complementary to the 5' region of AFP mRNA

A *Bam*HI-*Ava*I DNA fragment was isolated from the cDNA clone pAF6 (Fig. 1), annealed to mouse yolk sac mRNA and the resulting RNA/DNA template/primer used in the reverse transcriptase reaction to synthesize a cDNA copy for the 5' region of the mRNA template. Using the same reverse transcriptase the cDNA was converted to its double-stranded form¹⁶, treated with endonuclease *S*₁, tailed with about 15 (dC)-residues and annealed with the *Pst*I-linearized plasmid vector pBR322 which had been tailed with 15 (dG)-residues as previously described¹⁷. Clones of the *Escherichia coli* strain RR1¹⁸ which had been transformed¹⁹ by the annealed recombinant plasmids were identified by their tetracycline resistance and ampicillin sensitivity, the two markers being carried on the plasmid pBR322 genome¹⁸.

Clones containing sequences 5' upstream of the *Bam*HI-*Ava*I DNA primer were identified by colony hybridization²⁰; the probe for this hybridization was obtained by using polynucleotide kinase and [γ -³²P]ATP to end label²¹ the 5'-terminal *Pst*I-*Bam*HI DNA fragment of pAF6 (Fig. 1), containing the AFP cDNA sequence 5' upstream of the *Bam*HI-*Ava*I DNA primer. Sequence analysis of one of the positive clones, pAF197, which was found to contain about 400 base pairs 5' upstream of the last cDNA nucleotide of pAF6 (Fig. 1), revealed that this cDNA codes for the entire amino-terminal region of murine AFP up to amino acid -9 of the AFP prepeptide; a partial amino acid sequence of the prepeptide has recently been

reported²². As the two cDNA clones share an extensive (1.1 kilobase) segment of homologous DNA in their central portion and pAF6 codes for the 3'-terminal region of AFP mRNA, together they encode the entire sequence of the murine AFP molecule.

DNA sequence analysis of the AFP cDNA

In contrast to serum albumin, where the entire amino acid sequence is known for the human and bovine proteins, very little sequence information is available for AFP, apparently because of hindrance by the carbohydrate moieties of the molecule¹¹. What little sequence information is available includes 20 amino acids of the prepeptide²², some 20 residues at the amino terminus and the carboxy-terminal valine¹¹.

We obtained the amino acid sequence of AFP by determining the entire sequence of the AFP cDNA insert in pAF6 and pAF197 by the chemical method of Maxam and Gilbert²³, mainly using both DNA strands to ensure accuracy. All the restriction sites on the restriction map were accounted for by sequence analysis, and all the restriction sites used to end-label DNA fragments were sequenced across by labelling a neighbouring restriction site. The murine-derived sequence of pAF197 actually starts with the *Mn*II recognition sequence CCTC and corresponds to leucine -9 of the prepeptide. The sequence 5' upstream of leucine -9 was determined as described in Fig. 2 legend.

Figure 2 shows entire nucleotide sequence of the cDNA in pAF6, pAF197, of the primer-extended cDNA at the 5' terminus of the AFP mRNA and of the deduced amino acid sequence. The combined length is 2,064 nucleotides, of which 41 represent the 5'-untranslated region, 57 identify the prepeptide of 19 amino acids, 1,758 code for the 586 amino acids of AFP, 153 make up the 3'-untranslated region and 55 are the poly(A) sequence.

The hydrophobic presequence (Fig. 2) for AFP, which is 19 amino acids long, is processed to generate the mature polypeptide. The length and hydrophobic nature of this presequence are similar to those reported for other secretory proteins (see ref. 24 for a compilation of presequences). Instead of two terminal methionine residues²² only one is accountable for by direct DNA sequencing. No other ATG codons are observed in any reading frame in the entire DNA sequence 5' upstream from the amino-terminal methionine. Therefore, our data on the presequence show a length of 19 rather than 20 amino acids as previously reported²². The remaining DNA sequence data agree, with few exceptions, with the published amino acid sequence in this region of AFP. The amino-terminal residue in AFP is found to be Ala rather than the previously reported Ser¹¹. Considering that our sequence in this region is derived

Fig. 2 Nucleotide sequence of murine AFP mRNA as determined from cloned cDNA. The Maxam and Gilbert²³ method was used throughout this work for sequence determination. The sequence 5' upstream of leucine -9, which is not contained in pAF197, was determined as follows. A 0.1-kilobase *Hha*I(3,589)-*Hinf*I DNA fragment (Fig. 1) was isolated from pAF197 and 5' labelled with polynucleotide kinase²¹ and [γ -³²P]ATP. This fragment was subsequently digested with *Mn*II, which cuts at the very beginning of the AFP sequence and removes the pBR322 portion of the *Hha*I-*Hinf*I fragment. The resulting *Mn*II-*Hinf*I murine DNA fragment was labelled only at the *Hinf*I 5' terminus. This fragment was annealed to mouse yolk sac mRNA and the resulting RNA/DNA template/primer was used in the reverse transcriptase reaction to synthesize a cDNA copy extended into the 5' terminus of the mRNA template. This cDNA was separated from the deoxyribonucleotide triphosphates on a Sephadex G-50 column and used directly for sequence determination. The amino acid sequence shown is deduced from the nucleotide sequence. Nucleotides are numbered at the end of each row and amino acids above the sequence. In the 3'-untranslated region four potential termination codons are indicated in the amino acid reading frame. There seems to be no propeptide sequence in AFP analogous to that in serum albumin. The molecular weights for the aglycoprotein of AFP and its precursor, as determined from the amino acid composition, are 65,164 and 67,286, respectively.

The 5'-noncoding region of the murine AFP message reveals the presence of a purine-rich sequence in positions 26-38 (Fig. 2). This region exhibits partial sequence homology among

the various mammalian mRNAs which have been found to have a purine-rich region with its 3' end 2-6 nucleotides from the starting AUG codon (for a compilation of 5'-noncoding sequences see refs 25, 26). Nucleotides 6-14 (Fig. 2) are complementary to a 3'-terminal region of eukaryotic 18S

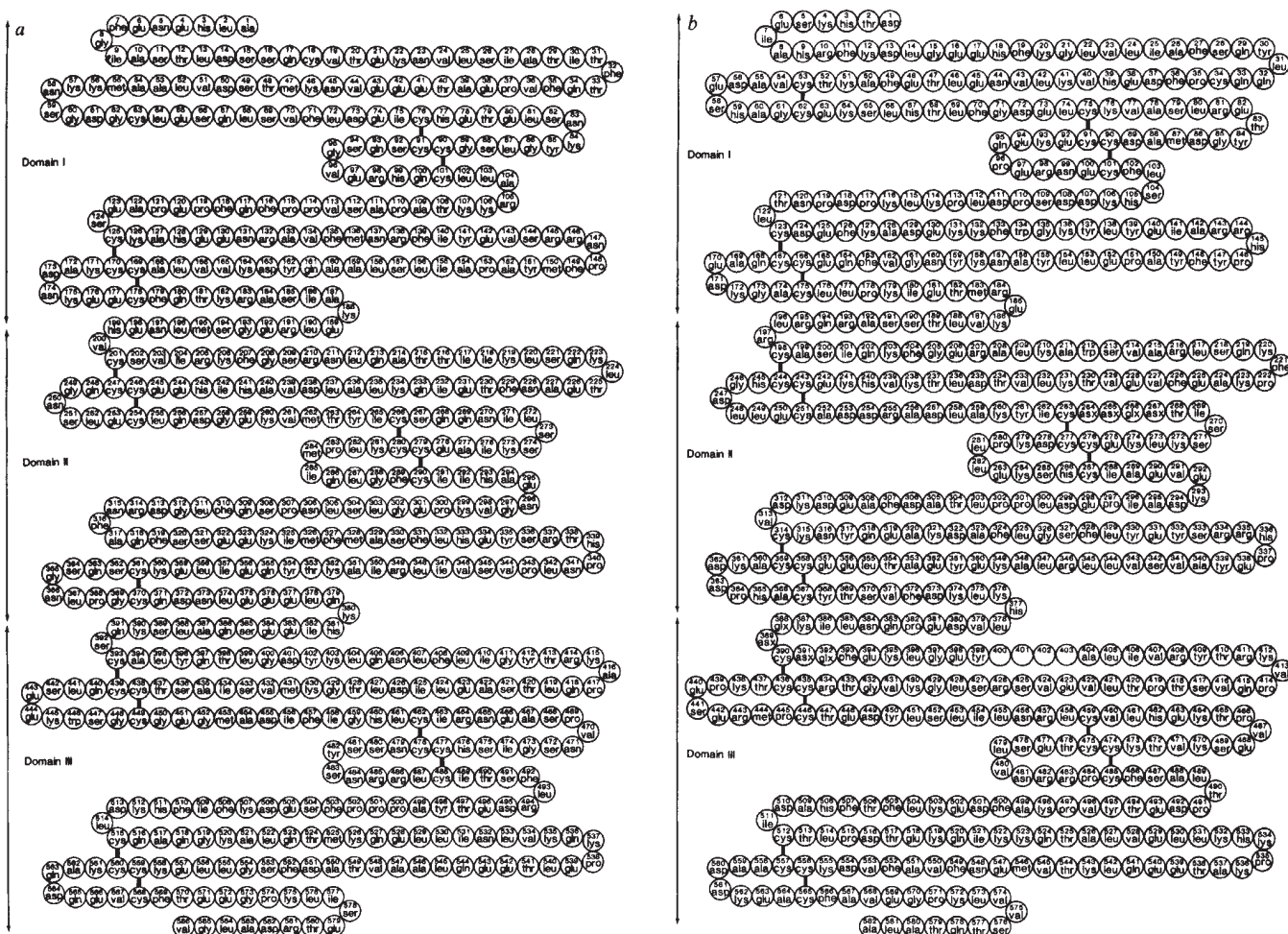


Fig. 3 *a*, Amino acid sequence and disulphide bonding pattern of murine AFP. A triple domain structure is indicated by arrows: I, amino acids 1–193; II, amino acids 194–385; III, amino acids 386–586. The sequence represents our present data, the layout of the sequence follows that of serum albumin, and the disulphide bridges are assigned by analogy with serum albumin. *b*, Amino acid sequence and disulphide bonding pattern of bovine serum albumin. The triple domain structure is indicated by arrows: I, amino acids 1–190; II, amino acids 191–382; III, amino acids 383–582. The sequence and its layout are according to Brown³³.

RNA²⁷ and thus could represent a ribosome binding site:



The 3'-noncoding region consists of 153 nucleotides beyond the carboxy-terminal valine. It starts with the translation stop signal UAA and contains four more potential stop codons in the same reading frame. The hexanucleotide AAUAAA, which is conserved in this region of eukaryotic mRNAs²⁸, is located in the AFP message 15 nucleotides 5' upstream from the poly(A). Contrary to reports on some human cDNA sequences²⁹, mouse AFP codons are not biased towards those ending in one or two specific nucleotides, although there is a bias in the use of certain codons.

Structural relationship between α -fetoprotein and albumin

From a detailed amino acid sequence analysis of bovine (BSA) and human (HSA) serum albumin^{30–33}, Brown discovered a repeating pattern in the structure^{32,33}. On the basis of spacing of the cysteine residues in the complete polypeptide chain and the positions of the disulphide bridges, the albumin sequence can be organized to generate three very similar repeat units, called domains (Fig. 3b).

To elucidate the structure of AFP, we have translated the entire AFP mRNA coding sequence into a corresponding amino acid sequence and aligned the disulphide bridges of cysteine

residues in the same way as has been described for BSA or HSA^{32,33}. The resulting structure reveals that the amino acid sequence of AFP can be divided into three domains of sequence homology, analogous to the domains of albumin (Fig. 3a, b). A total 15 disulphide bridges can be formed throughout the AFP molecule, two less than found in BSA. The cysteine residues occur within the polypeptide chain of AFP at almost identical sequence positions to those found in albumin, thus allowing for the formation of the same disulphide bridges in the secondary structure of AFP. Note however, the disulphide bridges in AFP are assigned here by analogy with serum albumin only, and we have no experimental evidence for such arrangement.

A comparison of the AFP amino acid sequence with that of human or bovine albumin shows about 40, 30 and 10% homology of identical amino acids between domains III, II and I, respectively. The homology between the two proteins increases further to ~65% for domain III and ~55% for domain II if the amino acids are compared on the basis of their belonging to one of four groups having a characteristic side chain: hydrophobic (Phe, Leu, Ile, Met, Val, Pro, Ala, Tyr, Trp), polar (Ser, Thr, Gln, Asn, Cys, Gly), basic (His, Lys, Arg) or acidic (Glu, Asp). Among the differences between the two proteins there are three potential N-glycosylation sites (Asn-X-Thr; Asn-X-Ser)³⁴ in the AFP sequence and none in albumin.

It is tempting to analyse the nucleotide sequence of the AFP message in the two regions where, in contrast to the albumin sequence, there are no disulphide bridges in the AFP structure. One such region is the Cys 53–Cys 62 disulphide pair in BSA

(Fig. 3b). In AFP, this structure corresponds to the juxtapositioned Ala 54–Cys 63 amino acid pair (Fig. 3a) which obviously lost a bridging capacity. There is, however, a cysteine UGC codon and thus a 'hidden' Cys residue in the critical position of Ala 54, if the reading frame is shifted by one nucleotide in this region (Fig. 2). A difference of one nucleotide is thus responsible for the difference of one extra disulphide bridge in the structure of the two proteins. The other region is the Cys 314–Cys 359 pair in BSA (Fig. 3b), which is also absent from the AFP structure, where it is replaced by the Ala 317–Ser 362 pair instead (Fig. 3a). Examination of the AFP nucleotide sequence (Fig. 2) reveals again that only single nucleotide changes are required to re-establish the missing Cys 317–Cys

362 structure in the AFP molecule. The mRNA sequence seems to carry a more detailed record of ancestral relationship between AFP and serum albumin than is evident from their existing amino acid homology. When DNA sequences for AFP and albumin from the same species become available, their comparison will probably give a better indication of the genetic relationship between the two proteins.

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Nucleotide sequence of *Xenopus laevis* 18S ribosomal RNA inferred from gene sequence

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18S ribosomal RNA in Xenopus laevis is 1,825 nucleotides long, as inferred from sequence analysis of an 18S gene. All the 40 rRNA methyl groups can be located in the sequence. Comparison with the yeast (Saccharomyces cerevisiae) 18S sequence reveals extensive regions of high homology interspersed with tracts having little or no homology. Regions of high homology contain almost all the RNA methyl groups. Major regions of low homology are considerably richer in C + G in Xenopus than in yeast.

KNOWLEDGE of the primary structure of ribosomal RNA (rRNA) in higher organisms should elucidate several aspects of ribosomal function, assembly and phylogeny. Here we report the nucleotide sequence of 18S rRNA in the frog *Xenopus laevis*, as inferred from analysis of an 18S gene. We compare our findings with the recently reported yeast (*Saccharomyces cerevisiae*) 18S sequence¹.

The sequence was determined from the *X. laevis* rDNA clone, pX1r 101, which contains a complete ribosomal transcription unit with its preceding non-transcribed spacer (Fig. 1). The sequencing strategy involved almost complete analysis of both strands of the 18S gene with extensive overlapping (Fig. 1); the inferred RNA sequence is shown in Fig. 2. Considerable evidence indicates that this sequence is representative of 18S rRNA in *X. laevis*. Oligonucleotide data on the 5' 160 nucleotides², the 3' 230 nucleotides (B.E.H.M., unpublished data), the various oligo(A) tracts³ and the methylated sequences (below) all agree with the DNA sequence. Limited sequence data from other clones of *X. laevis* rDNA agree with the sequence shown in Fig. 2 (B.E.H.M., unpublished results), and the many restriction sites identified here agree well with those reported previously for an independently cloned rDNA unit from *X. laevis*^{4,5}.

rRNA methylation

Knowledge of the sequence provides a means of determining the sites of all the rRNA methyl groups (40 in *X. laevis* 18S rRNA). Data on the methylated oligonucleotides in 18S RNA^{6–10} and on their approximate locations in the sequence as deduced from 'hybridization mapping'¹¹ have allowed us to locate all the methylated nucleotides. The methyl group distribution is highly irregular (see Figs 2, 3). There is considerable clustering of 2'-O-methyl groups in the 5' 40% of the sequence and the distribution in this region is also irregular; another cluster of 2'-O-methyl groups occurs in the region 1,240–1,400. The remaining 2'-O-methyl groups are outliers from the main clusters. All the base methyl groups are in the 3' 35% of the molecule. The hypermodified nucleotide am^ψ (1-methyl-3-aminobutyl pseudouridine¹²) is at position 1,209. m⁷G occurs at the point encoded by the EcoRI site (nucleotide 1,596). The base-modified A residues are in the last 40 nucleotides.

The numerous pseudouridines in 18S rRNA are also found by hybridization mapping to be widely distributed (unpublished results), but, as few have been precisely located², this class of modifications is not included in Fig. 2.

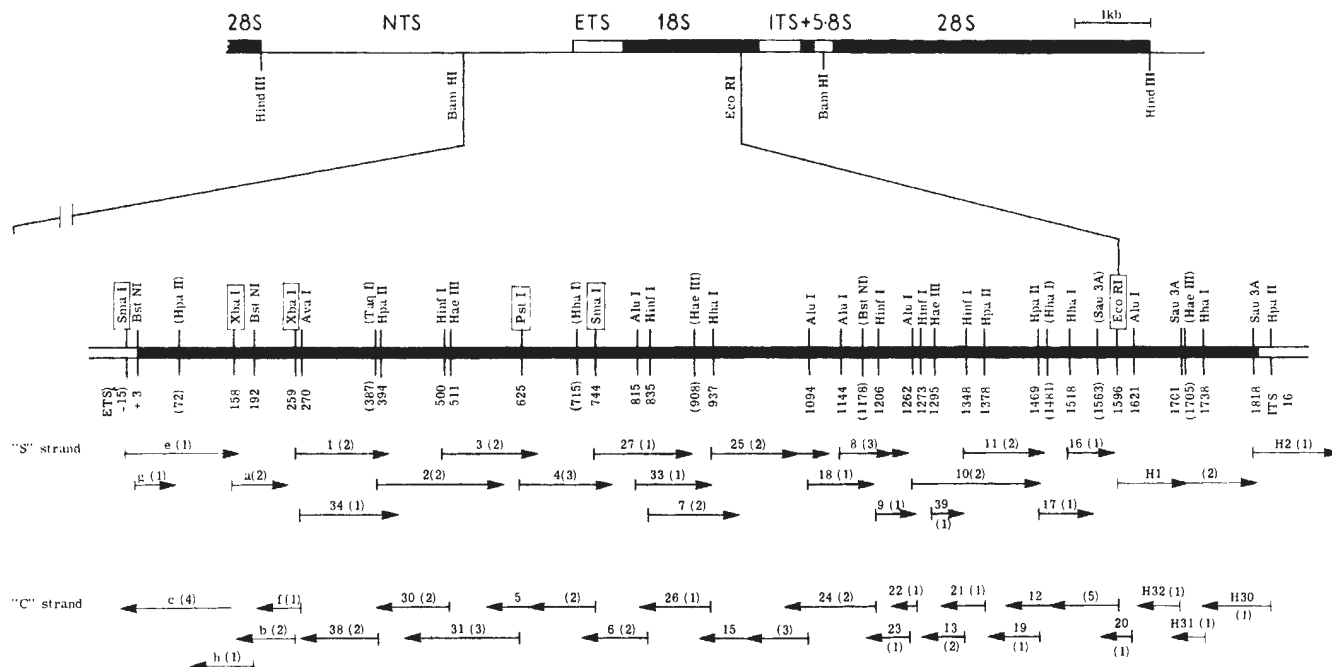


Fig. 1 Top line, repeating structure of rDNA in *X. laevis*. Solid sections represent rRNA coding sequences. NTS, ETS and ITS denote non-transcribed, external and internal transcribed spacers. pX1r 101 consists of a complete rDNA unit bounded by *Hind*III sites, cloned in the vector pMB9. Lower part of the figure shows the sequencing strategy. Most of the sequence was determined after subcloning the indicated 3.5-kilobase *Bam*HI/*Eco*RI fragment into pBR322 (ref. 2). The sequence to the right of *Eco*RI sites was determined after subcloning the 1.1-kilobase *Eco*RI/*Bam*HI fragment into pBR322 (ref. 30). The sequence through the *Eco*RI site was determined as indicated below (gel 20). Sequences of the 5' 275 nucleotides and 3' 227 nucleotides, including identification of the two ends of the 18S gene, have been reported elsewhere^{2,30}; sequencing strategy for these regions is included for completeness. The major part of the sequence was determined by the Maxam and Gilbert method³¹ from gels 1–38. Restriction sites used for sequencing are shown on the centre line; numbers denote the first nucleotide in each site counted from the 5' end of the 18S gene. Boxed sites were used separately or in combination for large-scale digests. Some of the resulting material was labelled at the 5' termini; the remainder was digested with one or more further enzymes before labelling. Upper arrows denote sequencing gels on the 'S' strand (synonymous to RNA); lower arrows denote gels on the 'C' strand (complementary to RNA). The tips of the arrows indicate the points to which the gels were read. Numbers in parentheses indicate the number of sequencing gels from each 5'-labelled starting point. When the distances that were read differed substantially between experiments (for example due to use of different secondary restriction sites) arrows with two tips are shown. The entire sequence was read on both strands except at the following points: restriction sites *Hinf*I 1,273, *Hpa*II 1,378 and *Alu*I 1,621 were sequenced only on S strand; sites *Xba*I 259 and *Eco*RI 1,596 were sequenced only on C strand. Secondary structure effects interfered with the reading of short segments on S strand at 244–7, 681–4, 1,146–7 and on C strand at 226–9, 666–7, 705–8. Methylated C residues in *Eco*RII sites were evident as gaps or very faint bands as follows: S4, C6; S804, C806; S1,180, C1,182. Gel 9 was unclear at 1,256 but at this and all other points referred to above, the sequence was unambiguously read on the other strand, and the restriction sites just mentioned were all experimentally demonstrated. (*Bst* NI cuts at methylated *Eco*RII sites.) In most cases the purification of fragments was straightforward, and sequencing was carried out after 5' labelling and secondary restriction. At a few points more complex procedures were needed to allow the necessary overlapping sequences to be obtained. The relevant gels were as follows. S strand—gel 18, partial *Alu*I digest yielded fragment 1,094–1,262. 5'-terminal labelling was followed by secondary restriction at *Hinf*I 1,206. Gel 17, fragment bounded by *Hpa*II 1,469 and *Eco*RI 1,596, was labelled and subjected to partial *Sau*3A digestion to yield fragment terminating at 1,563. (Another *Sau*3A site at 1,497 is not shown.) Gel 8 (*Alu*I fragment 1,144–1,262), gel 9 (*Hinf*I fragment 1,206–1,273) and gel 16 (fragment bounded by *Hha*I 1,518 and *Eco*RI 1,596) were sequenced after strand separation. C strand—Gel 23, *Alu*I fragment 1,144–1,262, was labelled and subjected to secondary restriction at *Bst*NI 1,179; gel 22, *Hinf*I fragment 1,206–1,273, was subjected to secondary restriction with *Hae*III at 1,229 (not shown). Gels 13 (*Hinf*I fragment 1,273–1,348) and 19 (*Hpa*II fragment 1,378–1,469) were subjected to strand separation. (In this and some other experiments only one strand was successfully purified.) Gel 20, this crucial fragment (*Alu*I 1,262–1,621), which allowed sequencing through the *Eco*RI site, was obtained from the parent plasmid pX1r 101 by *Alu*I digestion of a 4.5-kilobase *Bam* HI fragment (these sites are shown in the top line of the figure). The *Alu*I fragment was labelled, subjected to secondary restriction at *Hpa*II 1,469 and the C strand was sequenced. The result confirmed the presence of a single *Eco*RI site and the correctness of the adjacent sequences as previously deduced from the subclones.

Figures 2 and 3 show a comparison of the *X. laevis* and *S. cerevisiae*¹ sequences. There are extensive tracts where the two sequences are almost identical. Most of the two sequences are >70% homologous, but there are also tracts with little or no homology. Almost all the *X. laevis* methylation sites are in regions of high homology with the yeast sequence. Four major regions of low homology are designated *a*, *b*, *c* and *d* in Fig. 3. Table 1 shows that these regions are considerably richer in C + G in *Xenopus* than in yeast.

Evolutionary trends

These findings provide insight into evolutionary trends in eukaryotic rRNA and suggest a general explanation for a well-known paradox: rRNAs from distantly related eukaryotes differ considerably in overall base composition^{13,14} but show substantial homology by nucleic acid hybridization^{15,16}. Clearly in *Xenopus* and yeast 18S rRNA the nucleic acid hybridization experiments show extensive, highly conserved sequences. The divergence in base composition between the two rRNAs results from cumulative changes in less highly conserved parts of the molecules, and much of this variability is concentrated in clearly circumscribed tracts. These range in size from short runs of a few nucleotides such as tract 65–75 (Fig. 2) to the longer, major

variable regions of Table 1. Thus the pattern of sequence homology between *Xenopus* and yeast 18S rRNAs may be described as 'extensive but interrupted'.

How general is this pattern? Sequence analysis of the 3' regions (rightwards from the *Eco*RI site) of 18S genes in silkworm¹⁷ and fruitfly¹⁸ shows that much of this region has also remained highly conserved in these organisms, but again the tract corresponding to region *d* is more variable. Recent refinements in nucleic acid hybridization experiments, in which heterologous rRNAs are hybridized to specific restriction fragments of rDNA, confirm the general pattern of 'extensive but interrupted' homology for both 18S and 28S rRNA^{19,20}.

How variable are the variable regions? Table 1 shows that the 18S variable regions in *Xenopus* and yeast differ from each other less widely than do the respective internal transcribed spacers. The regions differ in length only slightly between the two species, and although the *Xenopus* regions are rich in G + C, the corresponding yeast regions are, on average, no more rich in A + U than the rest of the sequence (*a* is rich in A + U but *b* is slightly rich in G + C even in yeast). This suggests that there are constraints on evolution in these 18S regions, but not such stringent ones as in the highly conserved regions. The 18S variable regions possibly play a semi-specific structural role in

Table 1 *X. laevis* and *S. cerevisiae* base composition data

		No. of nucleotides	U	A	C	G	% C + G	
Complete 18S:								
	<i>X. laevis</i>	1,825	411	432	467	515	53.8	
	<i>S. cerevisiae</i>	1,789	509	475	347	458	45.0	
Major variable regions:								
	<i>a</i>							
	<i>X. laevis</i>	103	(176–278)	16	20	38	29	65
	<i>S. cerevisiae</i>	91	(177–267)	34	31	13	13	29
	<i>b</i>							
	<i>X. laevis</i>	110	(651–760)	20	10	43	37	73
	<i>S. cerevisiae</i>	106	(637–742)	35	12	29	30	56
	<i>c</i>							
	<i>X. laevis</i>	72	(1,351–1,395, 1,411–1,437)	13	15	25	19	61
	<i>S. cerevisiae</i>	71	(1,321–1,362, 1,378–1,406)	24	17	13	17	42
	<i>d</i>							
	<i>X. laevis</i>	75	(1,695–1,769)	11	14	26	24	67
	<i>S. cerevisiae</i>	71	(1,663–1,773)	17	19	15	20	49
	Sum							
	<i>X. laevis</i>	360	60	59	132	109	67	
	<i>S. cerevisiae</i>	339	110	79	70	80	44	
Rest of 18S:								
	<i>X. laevis</i>	1,465	351	373	335	406		50.6
	<i>S. cerevisiae</i>	1,450	399	396	277	378		45.2
ITS 1:								
	<i>X. laevis</i>	557	19	69	230	239		84
	<i>S. cerevisiae</i>	360	119	111	57	73		36

Numbers in parentheses indicate locations of the major variable regions in *X. laevis* and *S. cerevisiae*¹ 18S rRNA. Variable region c has two components separated by a short, conserved sequence with a methyl group in *Xenopus*. Data on ITS 1 are from refs 30 and 32.

Fig. 2 Nucleotide sequence of *X. laevis* 18S rRNA, with positions of methyl groups indicated by superscripts and comparison with the yeast *S. cerevisiae*¹ sequence indicated by subscripts. Most of the methyl groups were located from T₁ oligonucleotide data^{6-9,11}, but in several cases additional data from pancreatic ribonuclease fingerprints (B. E. H. M., unpublished results) were also needed—this work will be fully reported elsewhere. The methylation sites are as follows: m superscripts denote 2'-O-methyl groups at 27, 99, 116, 121, 163 (not 181 as was reported previously²), 393, 401, 427, 433, 449, 474, 477, 482, 541, 555, 566, 592, 609, one of 633-636, 648, 759, 761, 992, 1,249, Um and Gm between 1,280-1,289, 1,344, 1,352 or 1,355, 1,399, 1,447, 1,635, 1,660 and 1,760. Remaining superscripts denote base methyl groups at am ψ 1,209, m⁷G 1,596 (encoded at first base of *Eco*RI site), m⁶A 1,788, m²A 1,806 and 1,807. This list includes all known *X. laevis* 18S methyl groups except for one fractional 2'-O-methyl U which occurs at an unidentified location in the 5' third of the molecule. Also mentioned in the text are oligonucleotides containing oligo(A) tracts³. These are: A₄C, 218-222; A₅U, 484-489; (A₃, AmA)G, 633-638; A₆ ψ , 770-776; and A₄G, 1,778-1,782. Differences between the *Xenopus* and yeast sequences are indicated by plain subscripts in the case of substitutions; additional nucleotides in the yeast sequence are shown as subscripts between nucleotides in the *Xenopus* sequence and denoted by a circumflex sign. Crosses indicate deletions. In regions of low homology deletions are arbitrarily placed to allow optimal alignment between regions of good homology. (One apparent deletion from the yeast sequence occurs in a region of otherwise good homology with *Xenopus*, in nucleotides 1,006-1,015 of the latter. This part of the yeast sequence determination used a *Sau*3A site which does not seem to have been overlapped during analysis¹.)

puACCUGGUUG	AUCCUGCCAG	UAGCAU ^m AUGC	UUGUCUAAA	GAUUAAGCCA	UGCACGUGUA	AGUACGCCAG	GCCGUACAG	80
U		U			U C	UAAG A	AUUUA	
UGAAACUGCG	AAUGGCU ^m AU	UAAUACAGUU	AUGCU ^m CCUU	UGAUGCGUCC	AUCUGUUACU	UGGAUAACUG	UGGUAAUUCU	160
			C UA	A U	x U AC A	U C		
AGAGCUAAUA	CAUGCCGACG	AGCGCUGACC	CCCAGGAUG	CGUGCAUUUA	UCAGACCAAA	ACCAAUCCGG	GGCCCCCGCG	240
	UU AA	U U *	UUU A GA	GA U	U UA	AUC AUGU*	*****	
CCCCGGCCGC	UUUGGUGACU	CUAGAAUACC	UCCGCCGGAU	CGCAGUCCG	CGUGACGGCG	ACGAUACAUI	CGCAUGUCUG	320
UU A U	A U	AJA U	UxU GA	U G U	U CU	U G U	AA U	
CCCUAUCAAC	UUUGCAUGGU	ACUUUCUGCG	CCUACCAUGG	UGACCACGGG	UAACGGGGAA	UCAGGGUUCG	AU ^m UCCGGAGA	400
		GGA AGUG		UU A		A		
CGGAGCCUGA	GAAACGGCUA	CCACAU ^m CAA	GGAAGGCAGC	AGGCGCGCAA	AUUACCCACU	CCCCACGGCG	GGAGGUAGUG	480
					A	UA UU A		
ACGAAAAUAU	ACAAUACAGG	ACUCUUUCGA	GGCCUGUAA	UUGGAUAGAG	UACACUUUAA	AUCCUUUAAC	GAGGAUUAU	560
A U	G	G x CA UC	U U		A G	A C	A A	
UGGAGGGCAA	GUCUGGUGCC	AGCAGCCGCG	GUAU ^m UCCAG	CUCCAUAAGC	GUUAUUAUAA	GUUGCUGCAG	UUAAAAAGCU	640
						U		
CGUAGUUGGA	UCUUGGGUAC	GAGCUGGGCG	UCCGCCGCGA	GGCGGUACCC	GCCUGUCCCA	GCCCCUGCCU	CUCGGCGCCU	720
A	CU CC	***** UU	G GUC	UUUUUCGUG	UA GAUUU	C AA G GGC	UUC UU	
CCCCAGUAGU	CUUGACUGAG	UGUCGCCGGG	GCCCCAAGCG	UUUACUUUUA	AAAAUUUAGA	GUGUUCCAAG	CAGGCCGCGU	800
GG UA CCU	GAGUC UGU GGC	UU C	AA AGGA U			A	x UA	
CGCCUGGAUA	CUUCAGCUAG	GAUUAUUGGA	AUAGGACUCC	GGUUCUAUUU	UGUUGGUUUU	CGGAACUGGG	GCCAUGAUUA	880
U UC A	UA U AU	A	G UU		UA G C AUC	UA		
AGAGGGACGG	CCGGGGGCAU	UCGUUUUGUG	CCGCUAGAGG	UGAAAUUCUU	GGACCGGGCG	AAGACGAACC	AAAGCGAAAC	960
U	U	CG CAA	UU UC*		UUUAUUG	U U	CU	
CAUUUGCCAA	GAAUGUUUUC	AUUAUUAAG	AACGAAAGUC	GGAGGUUCGA	AGACGACUAG	AUACCGUGU	AGUUCGACCC	1040
G	G C	G	U	AG ****	***** U		CUUA	
AUAAACGAUG	CCGACUAGCG	AUCCGGCGCG	GUUAUUCCCA	UGACCCGCGG	AGCAGCUUCC	GGGAACCAAA	AGUUCUUUGG	1120
U	**	G U U	U UUA	A UC	G U C A	A U		
UUCCGGGGGG	AGUAUGGUUG	CAAAGCUGAA	ACUUAAAGGA	AUUGACGGAA	GGGCACCACC	AGGAGUGGAG	CCUGCGGCUU	1200
U	C	G			U		x	
AAUUUGCACUC	AACACGGGAA	ACCUCACCCG	GCCCCGACAC	GGAAAGGAUU	GACAGAUUGA	UAGCUUUUC	UCCAUUCUGU	1280
	G A A A	U A	U A			G	U U	
UmGmGGGUGGUGU	GCAUGGCGGU	UCUUAGUUGG	UGGAGCGAUU	UGUCUGGUUA	AUUCGGAUAA	CGAACGAGAC	UCCUCCAUUC	1360
	U	C	U	C	G	** Ux AC		
UAACUAGUUA	CGCGACCCCC	GGCGGUGCGC	GUCCAAUUC	UUAGAGGGAC	AAGUGGCGUU	CAGCCACAGC	AGAUCGAGCA	1440
CUA A G	U GUG UAG	AUUU CU U	UAU C		U UC UU CAAG	CGGAUGGAA	GUU GAG	
AUAACAGGUC	UGUGAUGCCC	UUAGAUUCC	GGGCGUGCAC	GCGCGCUACA	CUGAACGGAU	CAGCGUGUGU	CUACCCUGCC	1520
		A C U	U C		x x	GCCA C A	A UG	
CCGACAGGUG	CGGGUAAACC	CGUGAACCCC	GUUCGUGAUA	GGGACGGGG	AUUGCAUUA	UUUCCCAUCA	ACGAGGAUU	1600
G C	UU U U	UG A A U	CG C G	A A C	U	G U U C		
CCCAGUAAGU	GCGGGUCAUA	AGCUCGGGUU	GAUUAAAGUCC	CUGCCCUUUG	UACACACCGC	CCGUCGUAC	UACCGAUUGG	1680
U C	AA C	U	C			G	A	
AUGGUUUAGU	GAGGUCCUCG	GAUCGGCCCC	GCCGGGGUGG	GCCACGGGCC	UGGCGGAGCG	CCGAGAAGAC	GAUCAAACU	1760
C	C UCA	U UUA	AGAA GG	***** AA U	CAU UC A	G UUU	GA	
GACUAUCUAG	AGGAAGUAAA	AGUCGUAAAC	AGGUUUCCGU	AGGUUAACCU	CGGGAAGGAU	CAUUA		1825
GUC U G	C							

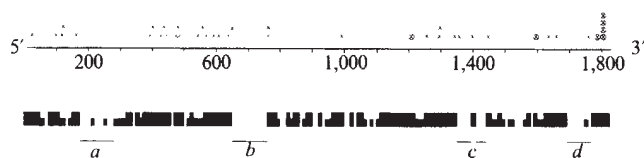


Fig. 3 Location of methylation sites in *X. laevis* 13S rRNA and pattern of homology with *S. cerevisiae*. Upper section: crosses denote 2'-O-ribose methyl groups; circled crosses denote base methyl groups. Lower section: high blocks indicate regions of *X. laevis* sequence possessing 85–100% homology with *S. cerevisiae*; low blocks indicate 70–85% homology. *a*, *b*, *c* and *d* denote major variable regions (see text and Table 1).

the ribosome. In 28S rRNA two long, variable regions do differ substantially in length between species^{21,22}.

What is the significance of localization of methyl groups within the conserved regions? Clearly many sites within these conserved regions provide recognition signals for methylases during ribosome maturation. However, further interpretation is difficult because the conservation of neither sequence nor methyl group numbers is absolute. (Yeast 18S rRNA possesses the same base methyl groups as *Xenopus* but some 15 fewer 2'-O-methyl groups^{23,24}.) Determination of the methylation sites in the yeast sequence should help to clarify the relationship between methylation and primary structure.

In contrast to the readily apparent and extensive tracts of homology between the *X. laevis* and yeast 18S sequences, we can detect little major homology with the *Escherichia coli* (prokaryotic) 16S sequence^{25,26} except near the 3' end. This lack

of extensive sequence homology between eukaryotic 18S and prokaryotic 16S rRNA is in agreement with results obtained from nucleic acid hybridization^{15,19} and T₁ ribonuclease oligonucleotide data²⁷. The relatively high degree of homology between the *Xenopus* and yeast sequences together with the lack of comparable homology between either of these and *E. coli* 16S rRNA further supports the widely held view that the divergence between the eukaryotic and prokaryotic systems for gene expression was a very ancient one^{27–29}.

Conclusions

Our conclusions and inferences on the structure and evolution of eukaryotic rRNA are as follows. (1) Substantial parts of the sequences have remained highly conserved since the last common ancestor of metazoa and yeasts, a period of probably 10⁹ yr. (2) Crucial events in the function or assembly of eukaryotic ribosomes, including rRNA methylation, centre on these conserved regions. (3) Tracts where primary structure is phylogenetically more variable are interspersed among the conserved regions. (4) The most clearly defined variable tracts are rich in C+G in *Xenopus*, and probably in vertebrates generally. (5) Phylogenetic change in these variable regions is less extreme than in the transcribed spacer regions.

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LETTERS

RG0044–2958: a peculiar M supergiant at a distance of 2.5 Mpc

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Peculiar M supergiants similar to VX Sgr and VY CMa are characterized by very red visual colours, a flux excess near 9,000 Å (*I* band) and a substantial excess longwards of 2 μm (refs 1, 2). HV11417 has recently been reported to be the first similar star discovered in the Small Magellanic Cloud³. We report here the discovery of another star of this type, RG0044–2958, which is more unusual in that it is located at a distance of ~2.5 Mpc, and is not associated with a detectable galaxy. It is probably the brightest star in a very low surface brightness dwarf galaxy in the Sculptor group.

We are presently studying the stellar distribution towards the South Galactic Pole to $m \approx 20$ in the *B*, *V*, *R* and *I* passbands, using UK Schmidt Telescope plates measured on the COSMOS facility at the Royal Observatory, Edinburgh. Our primary aim is the determination of the intrinsically faint end of the stellar luminosity function, and the solar neighbourhood space density of low mass stars. We use as a sensitive absolute magnitude calibrator for main sequence dwarfs the $M_V/V-I$ relationship derived directly from stars of accurately known parallax⁴. The resulting luminosity function can then be compared directly with that derived for the same field using classical kinematic techniques⁵. We are also observing the very red stars in the near IR (*JHK*) to allow luminosity class (dwarf/giant) and population type (halo/disk) discrimination.

One of the more interesting objects we have studied has the broadband energy distribution shown in Fig. 1, which also gives the energy distribution for the similar object HV11417 (from ref. 3), the two very late M dwarfs, VB8 and RG0050–2722 (ref. 6), and a 'typical' giant and supergiant⁷. The ability of *JHK* photometry to provide dwarf–giant classification is evident, while the substantial '*I*' band excess shows the object to be a peculiar M supergiant similar to HV11417 and VX Sgr. This identification has been confirmed from a spectrum obtained with

Table 1 Magnitudes for RG0044–2958

Passband	Mag
<i>B</i>	22.4±0.4
<i>V</i>	20.5±0.2
<i>R</i>	19.1±0.2
<i>I</i>	17.0±0.2
<i>J</i>	16.83±0.04
<i>H</i>	15.86±0.03
<i>K</i>	15.47±0.04

the multichannel scanner on the Palomar 5-m telescope (J.B. Oke and R. D. Cannon, personal communication).

There are two major differences between RG0044–2958 and VX Sgr: RG0044–2958 is not associated with a detectable galaxy, at least to the limit of the SRC 'J' Sky Survey, which has a limiting magnitude near $m_J = 23$, and it has not shown any substantial variability since 1976.7. There is, however, a strong 1-yr selection effect in our data, and any variability with a period near 1 yr would not have been detected. Useful images of RG0044–2958 appear on 15 USKT plates, of which five are IIIa-J(*B*), three are IIIa-F(*R*), four are *V* band and three are *I* band. It is not visible on either POSS print or the ESO 'B' Survey plate. The derived magnitudes are shown in Table 1, together with our IR (*JHK*) data. These were obtained with the 3.9-m Anglo-Australian Telescope on 27 October 1980. One of our deep *I* plates was obtained the following night, and precludes extreme variability as an explanation of the *I/R* colours. If RG0044–2958 does prove to be non-variable, the suggestion³

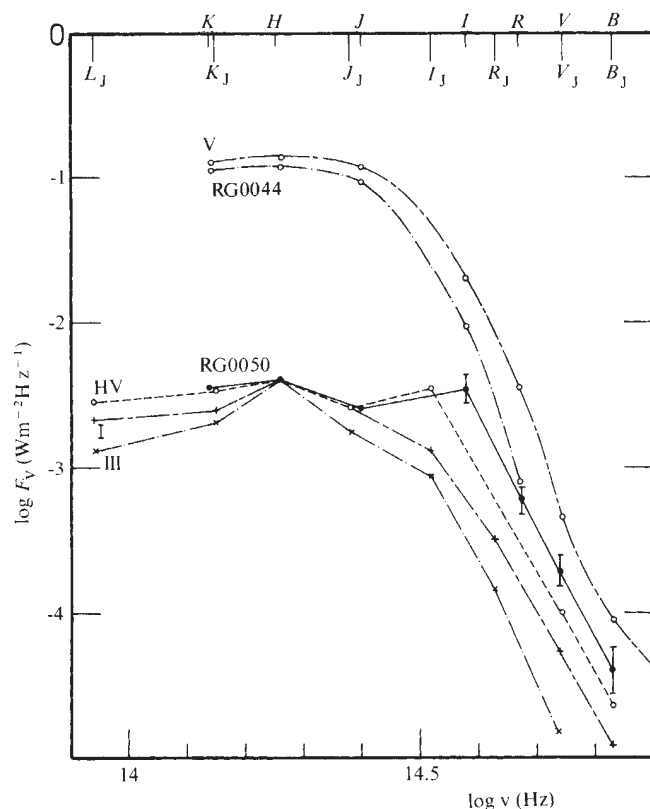


Fig. 1 Spectral energy distributions for the peculiar M supergiants RG0044–2958 (—), HV11417 (ref. 3) (HV, ---), and two late-type M dwarfs, VB8 (ref. 6) (V, ----) and RG0050–2722 (ref. 6) (— · — ·). A typical M5 giant (III) and supergiant (I) from the data of Lee⁷ are also shown. (The zero points are arbitrary.) The effective wavelengths of our filter passbands (*BVRIJK*) are marked at the top of the figure and are appropriate for our observations (RG0044, RG0050 and VB8). Those of the Johnson photometric system are marked with a subscript *J* (*BVRIJKL*) and apply to the observations of curves HV, I and III.

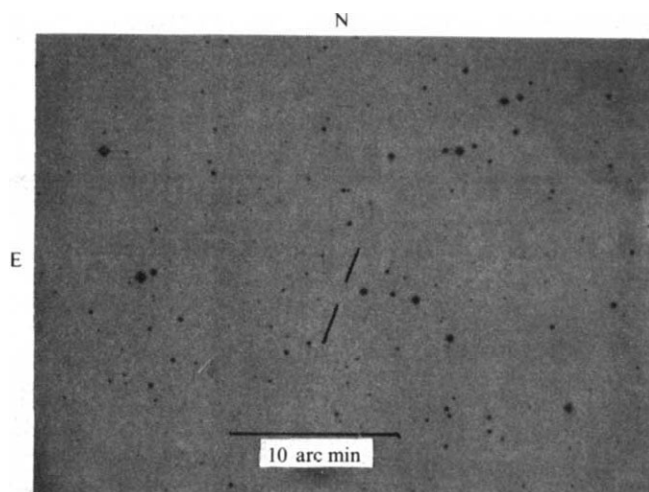


Fig. 2 A finding chart for RG0044–2958 reproduced from an *I* band UKST plate. North-east is at the top left corner, and the scale is marked. (Reproduced with permission from the UK Schmidt Telescope Unit, Royal Observatory, Edinburgh.)

that stars of this type are extreme long-period variables rather than peculiar supergiants would be disproved. The variable nature of the first three known stars of this type is readily explained as a selection effect, as the visual colours are fairly normal (for such late-type stars) and would not excite spectroscopic interest, whereas variability might. As seen in Fig. 1, either visual spectroscopy or both *I* band and near IR photometry are necessary to discover the peculiar nature of these stars.

The absolute magnitude of RG0044–2958 cannot be directly determined from our available data. Elias *et al.*³ derive a visual absolute magnitude $M_V = -5.1$ for HV11417, and note that the corresponding value for VX Sgr is only poorly known. Humphreys (Fig. 3 of ref. 8) shows the late M supergiants in M33 to have typically $M_V = -6.8$, with some as bright as $M_V = -8$. If RG0044–2958 is similar to these stars, it must have a distance modulus of some 27 mag, although this value may be uncertain by several magnitudes. Significantly, the Sculptor group of galaxies lies in the same direction in the sky, and has distance modulus 27.2 ± 0.2 mag with little if any reddening⁹. If this star is, in fact, associated with this group, its absolute magnitude becomes $M_V = -6.6 \pm 0.5$, consistent with the expected value. However, no associated galaxy is visible. The nearest Sculptor group galaxy is NGC253 (ref. 10), which is some 4.25° away. At a distance of 2.5 Mpc, this corresponds to a spatial separation of ~ 200 kpc. A velocity of $\sim 0.02c$ is then required for this star to have escaped from NGC253 in the lifetime of the precursor of an M supergiant. It is more likely that RG0044–2958 is the brightest star in a dwarf galaxy of low surface brightness, which is associated with the Sculptor group and possibly with NGC253 itself.

The position of RG0044–2958, determined relative to a grid of 76 SAO stars from the COSMOS measurements, is RA00 h 44 min 26.2 s, dec $-29^\circ 58' 49''$ (1950.0) and a finding chart is provided in Fig. 2.

As Fig. 1 shows there is no detectable difference between an extreme M supergiant and a very late M dwarf from broadband visual colours. The true number of objects similar to RG0044–2958 therefore cannot be determined until we have completed *JHK* photometry of our sample of very red stars.

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Solar radio blips and X-ray kernels

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Hudson¹ proposed that the primary flare energy release goes into fast electrons. Others^{2,3} found the flare soft X-ray emission to consist of bright, small kernels (knots and loops) embedded in a more diffuse halo. These kernels with a typical size of 5' arc s and a temperature of $6\text{--}12 \times 10^6$ K emit in radio waves at 3.7 and 11 cm (ref. 4). The discovery of a radio brightness temperature equal to the temperature derived from the X rays and the radio size larger than in X rays shows that the observed radio emission is thermal (free-free) and the source optically thick at $\lambda > 3.7$ cm. Therefore, Kundu *et al.*⁴ did not observe the fast electrons expected to heat the kernels. Here we present high time resolution measurements at 2.8 cm from the Effelsberg 100-m telescope and at decimetric and metric wavelengths with the ETH Zurich spectrometers. Both instruments have detected signatures of non-thermal particles.

We observed the microwave flux of McMath region 15856 on 2 March 1979. The Effelsberg telescope at 10.69 GHz yields a half-power beam width of 1.25 arc min. Simultaneous observations in the metric band by the Nançay heliograph and the Zurich digital spectrometer did not receive any signal in the 2-h observing period. However, decimetric registrations with the Zurich analogue spectrograph showed four groups of short, narrow-band spikes which coincided with the four largest microwave peaks of the observing period. This activity was also associated with four weak soft X-ray bursts as observed with the GOES 2 satellite. Figure 1 summarizes the data for one example. Note the following prominent properties of the radio emissions: (1) The time constant in decimetric and high-frequency microwaves is very small (2–3 s). (2) The radio bursts occur mainly in the rise phase of the soft X-ray emission (delay time of X-ray maximum 3–30 s). (3) The correlation between microwaves and decimetric radiation is excellent. (4) The amplitude of the microwave bursts is of the order of 2 SFU, much smaller than the bursts commonly observed by small telescopes monitoring the full solar disk and routinely reported in the flare bulletins.

These properties apply to all observed events and seem to be typical. All these events look very similar in microwave and X rays, but their shapes in the decimetric frequency-time plane show considerable variation. The flux ratio between single associated peaks in decimetre and microwaves fluctuates in a large range. The microwave emission has unusually short time scales and a very small amplitude. The bandwidth in decimetre waves is much smaller than commonly observed for type III bursts. These differences have led us to coin the name 'blip' for the radio emission.

No other activity has occurred in the decimetric and soft X-ray bands during that period. A chance coincidence is out of question. In microwaves, however, many smaller peaks have been observed with similar characteristics. Of the four blips to be

described here, three coincided with the maxima of subflares in McMath 15856.

A temperature of 10×10^6 K is derived for the plasma of the soft X-ray source from the ratio of the 1–8 Å and 0.5–4 Å intensities (correcting for line emission). The emission measure is $1.2 \times 10^{48} \text{ cm}^{-3}$. X-ray kernels have the tendency to avoid flares with non-thermal radio bursts^{2,3}. However, because the observed radio emission does not resemble impulsive radio bursts of conventional types and fluxes, we consider it very likely that the X-ray emission originates in kernels. This is supported by the fact that the observed temperature, emission measure and the correlation with subflares is exactly as in soft X-ray kernels². Thus we adopt a source size of 5×10 arc s and a volume of 10^{26} cm^3 . This yields a density of $\sim 10^{11} \text{ cm}^{-3}$.

The optical depth of the kernel at 10.7 GHz is 0.19, and the thermal radiation amounts to 0.67 SFU. Because the observed peaks are a factor of 2 or 3 higher and do not correlate with the X-ray burst, we conclude that the thermal radiation is absorbed in the vicinity of the source. A non-thermal origin for the microwave blips is also suggested from their correlation with their decimetric counterpart which, by its spectrum, is certainly not thermal. Thus we consider non-thermal electrons with velocities β (in units of the speed of light) estimated from the collisional loss time t_c , where

$$t_c = 3.3 \times 10^{12} \frac{(1 - \sqrt{1 - \beta^2})^2}{n\beta(1 - \beta^2)} \approx 3\text{ s}$$

with the ion density n in cm^{-3} . From the observed decay time of ~ 3 s one derives $\beta = 0.4$ corresponding to 50-keV electrons. The observed energy input of 2.76×10^{28} erg observed in X rays requires a total number of 4×10^{35} fast electrons. If the X-ray source is heated by the collisional energy loss of these electrons, a total number, N_T , of 4×10^{35} is required.

To estimate the gyrosynchrotron radiation of these electrons in a magnetic field B we applied the emission formula given by

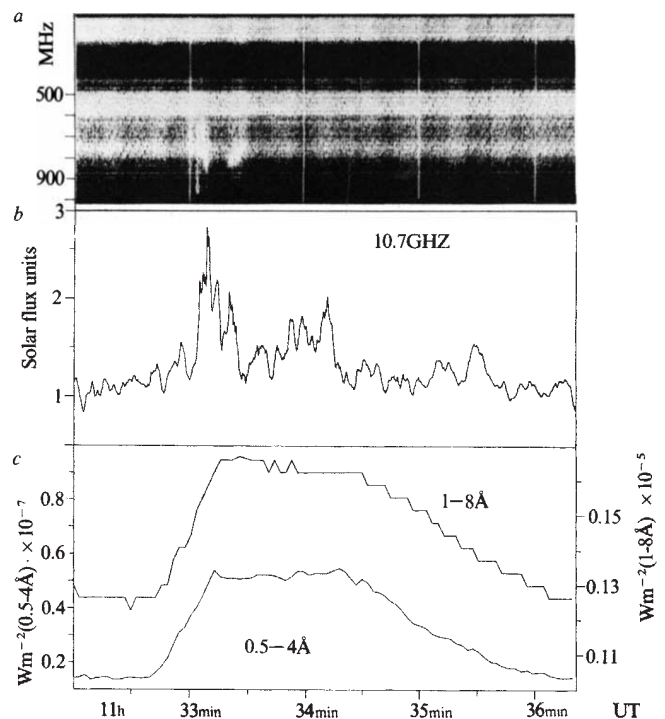


Fig. 1 Solar blip on 2 March 1979 in different wave bands. *a*, Spectrogram of the Zurich analogue spectrometer showing flux (bright) versus frequency (down) and time (right). Horizontal straight lines are all instrumental. The vertical lines are minute marks. The threshold is ~ 30 SFU. *b*, Total flux versus time observed by the 100-m telescope in Effelsberg (MPIfR, Bonn) of McMath region 15856. *c*, GOES 2 soft X-ray measurements in the bands 0.5–4 Å (left scale) and 1–8 Å (right scale).

Bekefi⁵ (formula 6.14). The number N of fast electrons present at any one time is their total number, N_T , times the ratio of the decay time and the total blip activity duration ($N = 6 \times 10^{34}$). From the observed average flux of about 0.5 SFU and from the estimated free-free absorption (source $\tau = 0.19$; foreground $\tau = 1.5$, as required from the lack of thermal radiation) we arrive at $B = 250$ – 300 G for low pitch angles and large angles ($>60^\circ$) between the line of sight and magnetic field direction.

In the total number of electrons estimated above we have not included fast particles that avoid energy loss in the X-ray source by escape to regions of lower density. Only a small fraction, however, is needed to produce the observed decimetric blip presumably by some plasma emission process.

In conclusion, according to the above discussion the microwave signal of non-thermal electrons is not to be expected at frequencies below 10 GHz. Therefore the previous observations by Kundu *et al.*⁴ probably refer to thermal emission of the hot plasma and not to fast particles. The discovery of decimetric signatures proves the presence of non-thermal particles in at least the larger microwave blips. Two important questions remain: first, is the observed rate of one blip (above 1 SFU at 10.7 GHz) in 25 min typical? Second, are the smaller fluctuations^{6,7}, which occur all the time, of the same nature? An extensive study involving major radio telescopes and the SMM satellite is in progress.

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New positive ion species in the stratosphere

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Our understanding of atmospheric ions in the ionospheric region is far better than of those in the denser regions, the stratosphere and the troposphere. Mass spectrometric measurements conducted on rockets¹ and balloons^{2–4} at altitudes above ~ 35 km recently revealed that the major ions are proton hydrates ($H^+(H_2O)_n$) and heteromolecular cluster ions identified as $H^+X_L(H_2O)_M$ (refs 2, 5) (X-ions), which probably are formed from proton hydrates by reactions involving a stratospheric trace gas X (ref. 2). The most likely candidate for X seems to be acetonitrile (CH_3CN) (refs 2, 6). The abundance ratio of X ions and proton hydrates increases with decreasing altitude from ~ 0.02 around 50 km to ~ 2 around 35 km. It has only been possible to detect ion species with number densities larger than about 6 cm^{-3} , corresponding to a fractional ion abundance of 3% around 35 km. We report here new balloon-borne positive-ion composition measurements around 34 km using improved mass spectrometers which allowed for an ion detection limit of $\sim 0.02\text{ cm}^{-3}$ or 0.1%.

The instruments used have been discussed in detail elsewhere^{2,5}. Improving the ion injection into the quadrupole mass filter and the efficiency of ion detection by the channeltron has increased the sensitivity. Thus, the overall ion transmission of the instrument was increased to $\sim 20\%$ depending on the mass

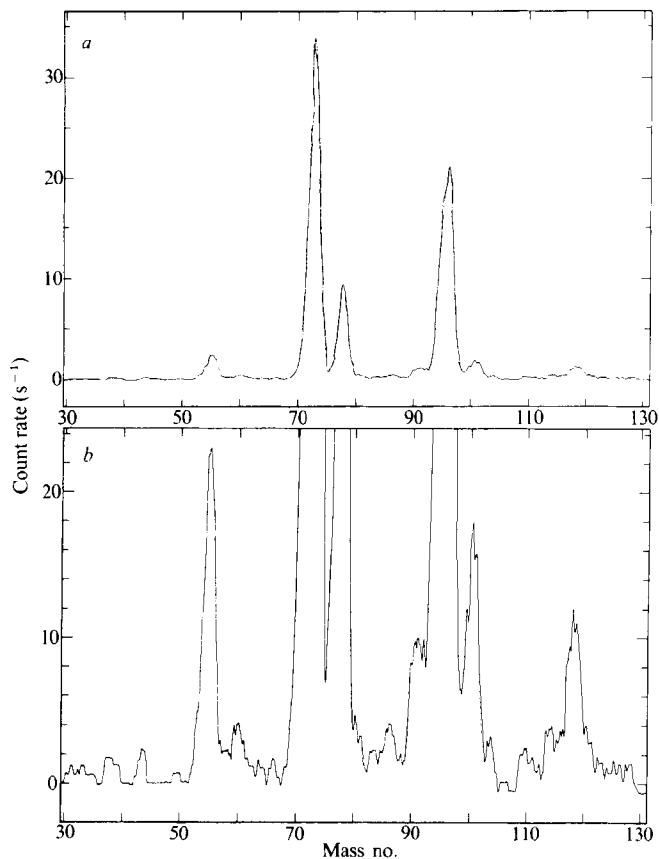


Fig. 1 *a*, Positive ion mass spectrum obtained at an altitude of 34 km (flight B 14). *b*, The same spectrum at an expanded scale for relative count rates. A relatively low mass resolution was used to reach higher sensitivity.

resolution setting. Usually, an increase of mass resolution causes a decrease of ion transmission, particularly at higher masses. Decreasing the dark count rate of the channeltron from ~ 0.02 to 0.001 s^{-1} contributed to an increase of the signal-to-noise ratio and thereby to the sensitivity for ion detection, as the channeltron is operated in a saturated pulse counting mode. For a sufficiently large integration time ($\sim 10^5\text{ s}$) a minimum detection limit of $\sim 0.002\text{ ions cm}^{-3}$ or 0.0001% can be reached for average mass resolution. Considering a more realistic integration time of only 10^3 s the detection limit becomes $\sim 0.2\text{ ions cm}^{-3}$ or 0.01% . If a more realistic minimum number of ion counts per mass peak of 10 rather than 1 were considered, the detection limit becomes $\sim 2\text{ ions cm}^{-3}$ corresponding to a fractional ion abundance of 0.1%. When compared with detection limits reached in stratospheric ion composition measurements reported previously, this value is lower by a factor of ~ 30 .

Three balloon-borne positive ion composition measurements were made around 34 km. Characteristic data of the experiments are given in Table 1. The mass range of the instruments which extended from 0 to 280 AMU was scanned in $\sim 11\text{ s}$ to allow for a high time resolution. Data measured at the same altitude were integrated during the data reduction by superimposition of a large number of individual mass scans.

The observed mass peaks may be divided into three groups having fractional count rates in the ranges: (1) 10–100%; (2) 1–10%; and (3) 0.1–1%. Group (1) includes the peaks 73, 78 and 96 AMU for all three flights; of these, either 73 or 78 dominate. Group (2) includes 55, 60, 91, 101 and 119 AMU. Among these, peaks 60 and 91 do not exceed the 1% level in all flights. All ions belonging to group (1) and (2) have already been detected previously and were identified as members of the ion families $H^+(H_2O)_n$ (55, 73, 91), $H^+X(H_2O)_i$ (60, 78, 96), and $H^+X_2(H_2O)_j$ (101, 119) (refs 1, 2, 5).

Table 1 Measured minor ion species having fractional count rates <1% and not belonging to the major ion families $H^+(H_2O)_n$ and $H^+X(H_2O)_n$

Ion	B9	B10	B14	$A^+(H_2O)$	$A^+(H_2O)_2$	$A^+(H_2O)_3$	$A^+X(H_2O)$	$A^+X(H_2O)_2$	A^+
45	+	+		<u>27</u>	<u>9</u>	—	—	—	27
49	+	+		<u>31</u>	<u>13</u>	—	—	—	31
58			+	<u>40</u>	<u>22</u>	4	—	—	22
63	+	+	+	<u>45</u>	<u>27</u>	9	—	—	27
67	+	+	+	<u>49</u>	<u>31</u>	13	8	—	31
81	+	+	+	<u>63</u>	<u>45</u>	27	22	4	27
86	+	+	+	<u>68</u>	<u>50</u>	<u>32</u>	27	9	32
104	+	+	+	<u>86</u>	<u>68</u>	50	45	27	27
110	+	+	+	<u>92</u>	<u>74</u>	56	51	<u>33</u>	33
117			+	<u>99</u>	<u>81</u>	63	58	40	63
122	+		+	<u>104</u>	<u>86</u>	<u>68</u>	<u>63</u>	<u>45</u>	63
125			+	<u>107</u>	<u>89</u>	71	68	<u>48</u>	48
128		+		<u>110</u>	<u>92</u>	<u>74</u>	69	51	74

Mass numbers for ion cores A^+ are also given assuming that the ions have the form $A^+(H_2O)_n$ or $A^+X(H_2O)_n$ with X having mass 41. The most probable core ions A^+ are underlined. All mass numbers given have an uncertainty of ± 1 AMU. The balloon flights B9, B10 and B14 took place over southern France on 30 April, 21 June and 26 September 1980 and reached float altitudes of 33, 34 and 34 km.

Several smaller peaks falling into group (3) can also easily be identified. They include 37 ± 1 , 42 ± 1 , 45 ± 1 , 49 ± 1 , 58 ± 1 , 83 ± 1 , 86 ± 1 , 104 ± 1 , 110 ± 1 and 114 ± 1 . The mass identification for these peaks is less certain as their shapes are not always well defined due to a small number of total ion counts contained in the peak. In other cases, small peaks are located besides large peaks and therefore appear only as shoulders. An example for a mass spectrum (flight B 14) is shown in Fig. 1. A mass uncertainty of ± 1 AMU must therefore be allowed for. Besides these 10 ions more members of group (3) were apparently detected in particular in the mass regions 60–73, 78–91 and 110–130. These peaks are only poorly defined and therefore uncertain. The count rates in these regions, however, are significantly above the background signal. Table 1 lists some peaks belonging to group (3) (out of 16) and indicates the flights in which the peaks were observed. Figure 2 shows the measured fractional ion count rates for the major families: (1) $H^+(H_2O)_n$; (2) $H^+X(H_2O)_n$; and (3) $H^+X_2(H_2O)_n$. The average numbers of ligand molecules are 2.7, 2.1, and 1.9. Evidently, incorporating X into the cluster ions decreases the average ligand number a result consistent with previous stratospheric measurements⁵ and laboratory studies^{6,7}.

The total fractional count rates for the above ion families are 48.2, 42.0 and 5.9%. Considering that families (2) and (3) are formed by incorporation of X into ions of families (1) and (2), the incorporation of a second X seems much less efficient than that of the first X. This contrasts with our previous measurement^{2,5}

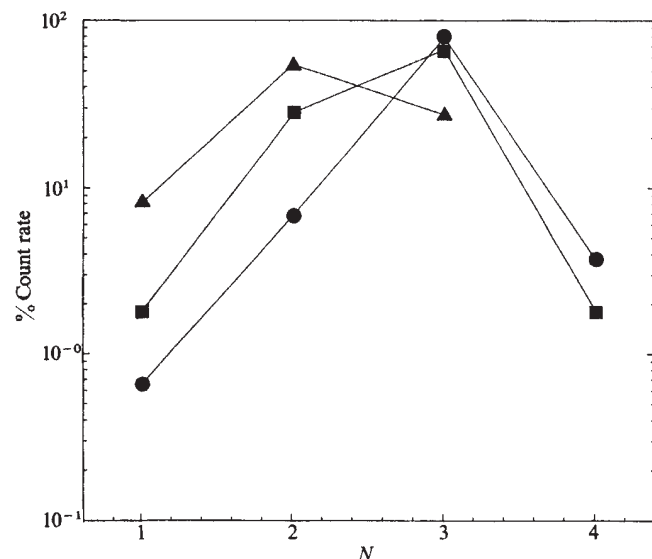


Fig. 2 Per cent count rates measured in flight B 14 for members of the ion families $H_3O^+(H_2O)_n$ (●), $H^+X(H_2O)_n$ (■) and $H^+X_2(H_2O)_n$ (▲) as function of the total number of ligands N .

which revealed that the efficiency for incorporation of one and two X molecules is about the same while that for the third X is much lower. The latter was interpreted in terms of backswitching of $H^+X_3(H_2O)_n$ ions with H_2O or of thermal detachment of an X from these ions^{5,6}. The present finding may suggest that due to the higher stratospheric temperature (240 K) during the B14 flight these processes even affected $H^+X_2(H_2O)_n$ ions.

Three out of the minor ions 37 ± 1 , 42 ± 1 , 83 ± 1 and 114 ± 1 may be readily identified according to their masses as H^+H_2O , $H^+X_1H^+X_2$ and $H^+X(H_2O)_4$ which are members of the main ion families. For 37 ± 1 , 83 ± 1 and 114 ± 1 this is supported by chemical arguments as the abundances of these ions fit very well the distributions shown in Fig. 2. The other minor ions (Table 1) do not belong to the major ion families. Like the X ions they are probably formed from proton hydrates by reactions involving trace molecules Y. They may be formed from X-ions if Y molecules have sufficiently large proton affinities. Thus, the reactions involving Y



and the subsequent reaction involving X



would give rise to the formation of ions having the general form $H^+Y(H_2O)_n$ and $H^+YX(H_2O)_n$. On their formation the product ions containing Y and possibly also X undergo rapid hydration and individual equilibrium distributions should be established for each ion family.

For ions of the types $H^+Y(H_2O)_n$ and $H^+YX(H_2O)_n$ the distributions should be similar to those for $H^+X(H_2O)_n$ and $H^+X_2(H_2O)_n$ ions (Fig. 2). This is also suggested by our laboratory measurements of cluster ion distributions which involved X and Y molecules as, for example, CH_3CN and CH_3OH (ref. 6). Consequently, if the minor ions listed in Table 2 have the form $H^+Y(H_2O)_n$ or $H^+YX(H_2O)_n$, they would be expected to contain two or three ligands. Species containing one and four ligands should be much less abundant and therefore were probably not detectable.

The total fractional abundances for the families $H^+Y(H_2O)_n$ and $H^+YX(H_2O)_n$ depend on the abundance of Y. A steady-state treatment of the formation of ions containing Y by processes (1) and (2) and of their loss by ion-ion recombination (rate coefficient α) yields

$$\{n(H^+(H_2O)_n + n(H^+X(H_2O)_n))\}kn(Y) = \{n(H^+Y(H_2O)_n) + n(H^+YX(H_2O)_n)\}\alpha n_- \quad (4)$$

where $n(\)$ denotes the number density of the species inside the bracket and n_- is the total negative ion concentration. Assuming that the minor ions are of the type $H^+Y(H_2O)_n$ or $H^+YX(H_2O)_n$

and considering that their total fractional abundance is of the order of 0.01, expression (4) yields $n(Y)$ of the order of $1,000 \text{ cm}^{-3}$. Here, k , α and n_- were taken as $2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$, $10^{-7} \text{ cm}^3 \text{ s}^{-1}$ and $2,000 \text{ cm}^{-3}$ (ref. 5). Thus, Y molecules seem to be ~ 100 times less abundant than X whose concentration is of the order of 10^5 cm^{-3} (refs 2, 5). Therefore, it is unlikely that ions containing more than one Y were detected. The total abundance of the $\text{H}^+\text{YX}(\text{H}_2\text{O})_n$ family should not exceed that of the $\text{H}^+\text{Y}(\text{H}_2\text{O})_n$ family by much more than a factor 2. Considering an X concentration of the order of 10^5 cm^{-3} , as inferred previously, the time scale for process (3) may be as small as the ion recombination lifetime.

By analogy to the X-ions the Y-ions might be expected to have the form $\text{HY}^+(\text{H}_2\text{O})_2$ and $\text{HY}^+(\text{H}_2\text{O})_3$. Mixed ligand clusters having the form $\text{HY}^+\text{X}(\text{H}_2\text{O})_n$ with n being mostly 1 or 2 may also be present in lower fractional abundances. It is also conceivable that instead of a protonated core H^+Y cluster ions contain an atomic core A^+ which has a low ionization potential as, for example, a metal atom. Assuming that the minor ions

Table 2 Tentative identifications for core ions, and proton affinities for protonated molecules

Mass	Core ion	Mass	Proton affinities (kcal mol ⁻¹)
23 ± 1	Na ⁺	23	—
27 ± 1	Al ⁺	27	—
	H ⁺ HCN	28	178.9 ~ 179
32 ± 1	H ⁺ CH ₃ OH	33	182
	H ⁺ CH ₃ NH ₂	32	217
48 ± 1	H ⁺ CH ₂ O ₂	47	178
	H ⁺ CH ₄ S	49	186
	H ⁺ C ₂ H ₆ O	47	187
63 ± 1	H ⁺ CH ₃ NO ₂	62	188
74 ± 1	H ⁺ C ₂ H ₃ NS	74	187

listed in Table 1 contain two or three ligands which may be H₂O or an X molecule and H₂O molecules mass numbers for the ion cores may be inferred (see Table 1). As the same core may be contained in more than one of the detected ion species the identification of a particular core becomes increasingly likely the more ion species that can be related to it. Unfortunately, however, because some of the minor ions are also sufficiently abundant to be detectable they may be masked by large adjacent mass peaks which complicates the grouping of ion species into families.

The minimum number of cores that the 14 minor ion species listed in Table 1 may be related to is 6, including the core masses 23 ± 1, 27 ± 1, 32 ± 1, 48 ± 1, 63 ± 1 and 74 ± 1. However, because there are alternative mass identifications of cores the core masses inferred above are not unique. For example, ions 63 ± 1 and 81 ± 1 which may be hydrates of a core 27 ± 1 and contain two and three water ligands were found in flight B14 to have fractional count rates (relative to the total count rate of this family) of ~ 25 and 75% . These values are almost the same as those of the corresponding hydrates of H⁺X (see Fig. 2) which supports the above identification for 63 ± 1 and 81 ± 1.

The ion composition data previously obtained by rocket-borne mass spectrometers were affected by strong collision-induced cluster ion dissociation and had revealed the presence of an ion 29 ± 2 which has not yet been satisfactorily explained. This ion species may, in fact, be identical to the above ion core 27 ± 1.

Tentative identifications for the above ion cores are given in Table 2. No gaseous compound from which the cores may have formed has so far been detected in the stratosphere. Although CH₃OH and CH₃NO₂ have been considered as stratospheric trace gases⁹. The core ion 27 ± 1 may also be Al⁺. However, Al⁺ reacts efficiently with O₃ to give AlO⁺ (F. Fehsenfeld, personal communication) which makes it an unlikely candidate.

Contaminant gases desorbing from the balloon or the payload may reside for a relatively long period of time in the vicinity of

the payload as this moves only at low speed ($1\text{--}10 \text{ cm s}^{-1}$) relative to the atmospheric gas. Thus, the payload may, in fact, be surrounded by a cloud of contaminant gases containing compounds which have the potential to react with atmospheric ions approaching the payload. In this respect, balloon-borne ion composition measurements differ greatly from rocket-borne measurements in the stratosphere which should not be affected by contamination as relative velocities are of the order of 1 km s^{-1} and thus contaminants should be carried away from the mass spectrometer by the gas flow. Therefore, ions detected in rocket-borne measurements should at least contain the same ion cores as the ambient stratospheric ions. As mentioned before, they may, however, have lost ligand molecules due to collisional dissociation.

The ions 42 ± 2 and 29 ± 2 detected by the rocket measurements should, therefore, represent cores of stratospheric ions. Whether other core ions inferred from the present measurements are also unaffected by contamination cannot be decided.

Future balloon-borne ion composition measurements should therefore be carried out in conditions of rapid motion of the probe relative to the atmospheric gas which reduces the chance that atmospheric ions collide with contaminants. Technically, this can be achieved by using a balloon which descends at a controlled speed of about 1 m s^{-1} which still allows for sufficient integration time.

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Carbon dioxide, ammonia and the origin of life

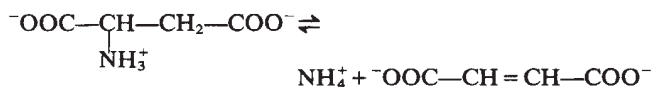
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Stellar evolution theory predicts that the luminosity of the Sun has increased by $\sim 30\%$ over the past 4,000 Myr. Yet geological and biological evidence indicates that the climate of the Earth between 3,000 and 4,000 Myr ago was as warm as, or warmer than, today. This apparent contradiction, the 'faint Sun paradox', has been resolved by invoking the greenhouse effect of radiatively active gases in the early Earth atmosphere. Sagan and Mullen¹ first suggested that the concentration of ammonia in the early atmosphere was around 10–100 p.p.m., sufficiently high to counteract the reduced luminosity. However, because ammonia photodissociates readily and has a short atmospheric residence time^{2,3}, such a concentration could be maintained only by a large continuous ammonia source. For this reason, carbon dioxide is now considered to have been the radiatively active gas^{4–7}. Some atmospheric ammonia is, nevertheless, required to provide conditions conducive to the origin of life⁸. We now show that, if the early Earth's atmosphere contained high concentrations of CO₂, as suggested above, then the chemical conditions required for life to begin can be maintained by very low ammonia partial pressures, rather similar to those observed today.

The conditions believed necessary for life to have evolved on Earth are: the existence of liquid water; and aqueous chemical conditions which permit a stable solution of simple amino acids, the chemical precursors of life. Organic molecules may form in the absence of ammonia, but ammonia is probably essential to ensure the stability of amino acids. Amino acid stability depends on temperature, pH and the concentration of dissolved NH_3 (ref. 8). The early evolution of life, therefore, places constraints on the chemistry of early aqueous environments, and hence on the atmospheric concentration of ammonia. To quantify these constraints, we will consider two end-member aqueous systems, a seawater and a freshwater, as possible life-origin environments. First, however, we will determine a general lower bound to aqueous ammonium ion activity as a function of pH and P_{NH_3} .

A minimum ammonium ion activity is required to prevent the decomposition by reversible deamination of amino acids to the corresponding hydroxy acids. Bada and Miller⁸ consider the deamination of aspartic acid to fumaric acid



$$pK = -8.22 + 2.276/T + 0.0106T; pX = -\log_{10} X, T \text{ in K} \quad (1)$$

and suggest that a necessary condition for life to begin is that the ratio of total aspartate to total fumarate should not fall much below one. At near-neutral pH the aspartate-fumarate ratio sets a lower limit to the ammonium ion activity of around $10^{-2.6}$ (at 25 °C). At lower pH values, higher ammonium ion concentrations are required to offset the higher stability of fumarate to protonation. From equation (1) and the acidity constants for aspartic and fumaric acids we have

$$\text{Asp}^{\text{TOT}}/\text{Fum}^{\text{TOT}} \approx a\text{NH}_4^+ 10^{-pK} \alpha_a / \alpha_t \quad (2)$$

Here

$$\alpha_a = \frac{K_a'''}{a\text{H}^+} + 1 + \frac{a\text{H}^+}{K_a''} + \frac{(a\text{H}^+)^2}{K_a'} \quad \text{and} \quad \alpha_t = 1 + \frac{a\text{H}^+}{K_t''} + \frac{(a\text{H}^+)^2}{K_t'}$$

where K_a''' , K_a'' and K_a' are the dissociation constants for aspartic acid, K_t'' and K_t' those for fumaric acid, and $a\text{H}^+$ ($\equiv 10^{-\text{pH}}$) is the hydrogen ion activity. At 25 °C, $pK_a''' \approx 9.9$, $pK_a'' \approx 3.8$, $pK_a' \approx 1.8$, $pK_t'' \approx 4.4$ and $pK_t' \approx 7.4$. Equation (2) is obtained from the acidity reactions of aspartic and fumaric acids neglecting the effect of ionic strength on the activities of the organic acid species. The stability constants quoted are from ref. 10. For $5.3 \leq \text{pH} \leq 8.4$, $\alpha_a \approx 1$, and for $\text{pH} \geq 5.4$, $\alpha_t \approx 1$. The isopleth $\text{Asp}^{\text{TOT}}/\text{Fum}^{\text{TOT}} = 1$ (at 25 °C), curve (1) on Fig. 1, places an approximate lower limit on the ammonium ion activity for life to evolve.

Equation (2) and the constraint $\text{Asp}^{\text{TOT}}/\text{Fum}^{\text{TOT}} = 1$ determine a minimum ammonium ion activity as a function of pH. The corresponding ammonia partial pressures are determined by the dissociation reaction of NH_4^+ . From the mass action equation for this reaction we have

$$pP_{\text{NH}_3} = pK_a - pK_{\text{H},a} + p(a\text{NH}_4^+) - \text{pH} \quad (3)$$

where $K_{\text{H},a}$ is the Henry's law constant for ammonia ($pK_{\text{H},a} = -1.785 - 34.85(298.15 - T)/5.7118 T$, based on free energy and enthalpy data in ref. 11) and K_a is the dissociation constant for NH_4^+ ($pK_a = 0.6322 - 0.001225 T - 2835.76/T$; ref. 12). Lines of constant ammonia partial pressure at 25 °C based on this equation are shown in Fig. 1.

We now consider the case where life began in a seawater environment in which chemical conditions were controlled by large-scale factors. In these circumstances, an extreme upper limit to the ammonium ion concentration may be set by dissolving all the available atmospheric nitrogen in the oceans as ammonia⁸ (curve (2) in Fig. 1). A more realistic upper limit has been suggested⁸ based on the cation exchange properties of clay minerals (curve (3) in Fig. 1). The zone between curves (1) and (3) in Fig. 1 defines the range of suitable ammonia partial

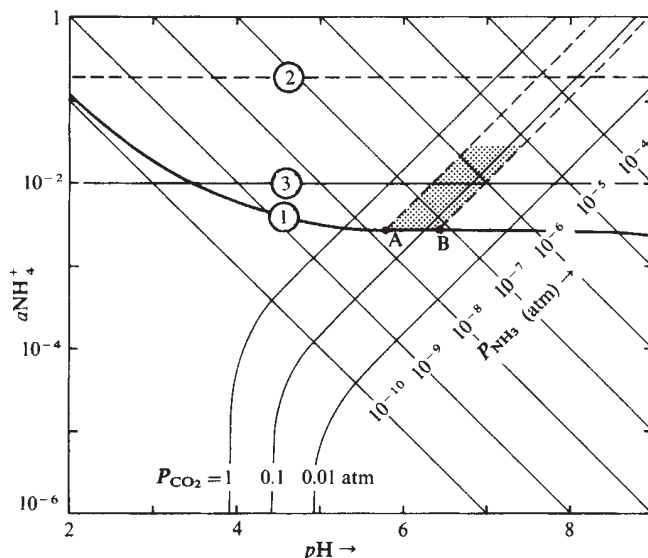


Fig. 1 Curve (1) and lines (2) and (3) define approximate lower (1) and upper (2 or 3) boundaries for ammonium ion activity as a function of pH for the amino acid stability required to allow life to evolve. The ordinate gives ammonium ion activity on a log scale. Isopleths of constant P_{NH_3} are shown as diagonal lines sloping from left to right calculated using equation (3). The zone between curves (1) and (3) is that which would be suitable for life to begin in an oceanic environment. At moderate pH (≤ 6), suitable conditions would prevail even for low P_{NH_3} ($\leq 10^{-8}$ atm). In a freshwater environment, chemical conditions are additionally influenced by P_{CO_2} . Curves of constant P_{CO_2} are also shown (based on equation (8)). These relate the four variables, P_{CO_2} , P_{NH_3} , $a\text{NH}_4^+$ and pH in freshwater environments. A 'life zone' (stippled region) is defined by the range of P_{CO_2} partial pressures thought to have prevailed between 3,500 and 4,250 Myr BP (ref. 20), and by the amino acid stability curve (1). This figure is based on thermodynamic data for 25 °C.

pressures for life to evolve. pH is the main controlling factor. As the early oceans were probably much more acidic than today¹³, extremely low ammonia partial pressures (as low as 10^{-8} atm at pH 5.6, see Fig. 1) would be sufficient to ensure a ratio of aspartate to fumarate in excess of 1. Present-day values, while subject to some uncertainty due to measurement difficulties, are of the order of $2\text{--}6 \times 10^{-8}$ atm over ocean areas^{14,15}.

There is, however, no *a priori* reason to assume that life originated in the early oceans. We must, therefore, consider the small-scale end-member possibility, a local 'fresh' water environment. Here the high atmospheric carbon dioxide partial pressures thought to have prevailed at the time are likely to have controlled pH values. To evaluate the effect of high P_{CO_2} on pH we consider a simple model in which only ammonium and carbonate species are present. Charge balance necessitates that

$$m\text{NH}_4^+ + m\text{H}^+ = m\text{OH}^- + m\text{HCO}_3^- + 2m\text{CO}_3^{2-} \quad (4)$$

where m denotes molality. For $P_{\text{CO}_2} \geq 10^{-5}$ atm, $\text{pH} \leq 9$ and $P_{\text{NH}_3} \geq 10^{-10}$ atm, ammonium and bicarbonate ions are the dominant species and the charge balance equation becomes

$$m\text{NH}_4^+ = m\text{HCO}_3^- \quad (5)$$

Within the accuracy of this result molalities may be replaced by activities, as the activity coefficients of NH_4^+ and HCO_3^- , as determined by the extended Debye-Hückel formulation, are approximately equal. Hence

$$a\text{NH}_4^+ = P_{\text{CO}_2} K_{\text{H},c} K_c / a\text{H}^+ \quad (6)$$

This result comes directly from equation (5) using $a\text{H}_2\text{CO}_3^* = K_{\text{H},c} P_{\text{CO}_2}$ where $pK_{\text{H},c} = 14.0184 - 0.015264 T - 2385.73/T$ is the Henry's Law constant for carbon dioxide¹⁶, and the mass

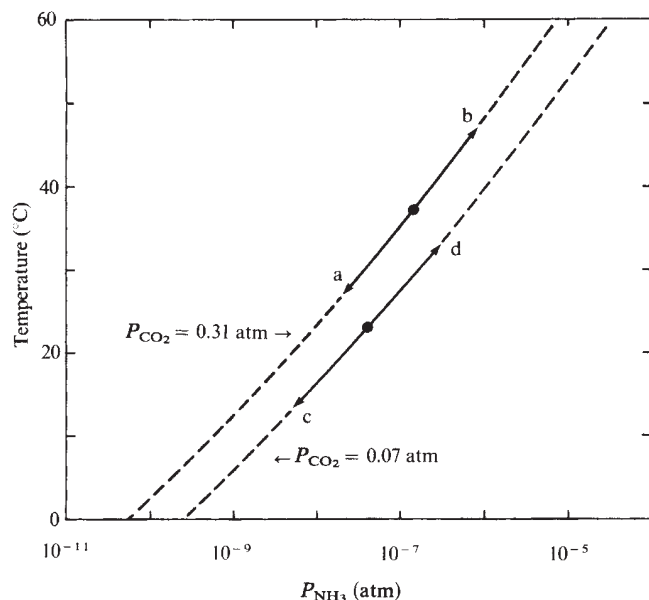


Fig. 2 The effect of changing temperature and P_{CO_2} on the minimum ammonia partial pressure needed for life to evolve. The points a and b span conditions which are thought to have prevailed around 4,250 Myr BP, while c and d span conditions for 3,500 Myr BP. If temperature is lower, then lower P_{NH_3} values are sufficient. The coupling of temperature and P_{CO_2} through the greenhouse effect means that life could have originated at lower P_{NH_3} for lower P_{CO_2} (see points a and c), in spite of the fact that the direct influence of P_{CO_2} is in the opposite direction.

action equation for the dissociation of H_2CO_3^* , $\text{aHCO}_3^- \text{aH}^+ = K_c \text{aH}_2\text{CO}_3^*$ where $pK_c = -14.8435 + 0.032786 T + 3404.71/T$ (ref. 16). H_2CO_3^* is the sum of dissolved carbon dioxide and carbonic acid. Equation (6) can be written in logarithmic form as

$$pP_{\text{CO}_2} = -pK_c - pK_{\text{H}_2\text{O}} + p(\text{aNH}_4^+) + p\text{H} \quad (7)$$

which allows isopleths of constant carbon dioxide partial pressure to be constructed on Fig. 1. Strictly this linear relationship is only valid for $P_{\text{NH}_3} \geq 10^{-10}$ atm. Although lower ammonia partial pressures are not relevant here the more general relationship, obtainable from equation (4),

$$10^{-p\text{H}} + \text{aNH}_4^+ \approx 10^{p\text{H}} [10^{-14} + 10^{-7.8} P_{\text{CO}_2} (1 + 10^{-10+p\text{H}})] \quad (8)$$

has also been used in constructing Fig. 1. In deriving equation (8) activity coefficients have been neglected (compare with derivation of equation (7)), but this results in no significant loss of accuracy.

Life is believed to have originated within the Earth's first billion years, possibly earlier than 3,000 Myr ago (ref. 17; but see also refs 18 and 19). Hart²⁰ estimates that the atmospheric P_{CO_2} was 0.07 atm at 3,500 Myr BP and 0.31 atm at 4,250 Myr BP, and values of this magnitude are required to give a sufficient greenhouse effect to compensate for reduced solar luminosity⁴⁻⁷. The range 0.07–0.31 atm P_{CO_2} therefore determines the range of pH values in early freshwater environments. Combined with the lower limit to aNH_4^+ derived earlier this range defines a 'life zone' in Fig. 1 (stippled region) within which chemical conditions would have been conducive to the origin of life. Note that the upper limit imposed by cation exchange considerations (curve (3) in Fig. 1) need not apply to the freshwater environment.

Figure 1 shows immediately that for life to evolve the local P_{NH_3} pressure must exceed 10^{-8} atm. Present-day values in non-oceanic areas are usually less than this value, but may exceed it. Values quoted recently range between 0.1 and 1.2

10^{-8} atm. (refs 14 and 21), and even higher values have been reported²². All these data may reflect local organic processes and so are not strictly relevant to conditions which might have prevailed before the origin of life. However, similar levels could easily be attained by the catalytic photochemical reduction of atmospheric nitrogen by TiO_2 in desert sands. Henderson-Sellers and Schwartz²³ postulate a 100-fold increase above present productivity by this mechanism to estimate a total atmospheric ammonia loading of 10^{13} kg, which corresponds to a partial pressure of around 1.9×10^{-6} atm. Using their figures, and present productivity levels, gives an estimated global atmospheric ammonia mass of 7×10^{10} – 7×10^{12} kg, equivalent to a partial pressure between 1.3×10^{-8} and 1.3×10^{-6} atm. While these values may be a little high compared with present-day measurements^{14,21} the lower end seems reasonable for local environments.

Figure 1 only shows the results for 25 °C, but we have performed the same calculations at other temperatures. The effect of temperature is shown in Fig. 2 where the ammonia partial pressure minimum spanned by the freshwater 'life zone' in Fig. 1 (points A and B) is plotted as a function of temperature. As temperature decreases the minimum ammonia partial pressure decreases. Mean global temperature estimates corresponding to the limiting P_{CO_2} values of 0.31 and 0.07 atm assumed above have been given by Owen *et al.*⁶. Assuming a global temperature range of ± 10 °C we can define regions in Fig. 2 which correspond to the range of P_{CO_2} and temperature values likely for the early Earth. If life began in a low salinity aqueous environment (such as might exist where local conditions today are conducive to the inorganic production of ammonia) then local P_{NH_3} values around 10^{-7} atm and possibly as low as 10^{-8} would have been sufficient to have maintained a suitable ratio of amino to hydroxy acids. Global mean P_{NH_3} may well have been even smaller.

Life may have been originated in highly saline conditions; the seawater case considered initially. In this case low ammonia partial pressures ($\leq 10^{-8}$ atm) are still sufficient, provided the pH is relatively low ($\text{pH} \leq 6$) compared with modern oceanic environments. Thus, high partial pressures of ammonia are not essential precursors for the origin of life in either of the systems considered here. Although our P_{NH_3} estimates are substantially less than some other estimates ($\sim 10^{-4.5}$ atm, see refs 1, 20, 24), and much less than the limit ($\sim 10^{-5}$ atm) for which a noticeable greenhouse effect might occur^{3,23}, such values seem unlikely because of the rapidity with which ammonia is photodissociated^{2,3}. Our values are similar to values which occur locally today. A strongly reducing atmosphere does not seem to be necessary for the origin of life.

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Archaeomagnetic secular variation record of Mount Vesuvius

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The lavas produced by historically recorded eruptions of Mt Vesuvius, and the Herculaneum mudflow resulting from the AD 79 eruption have been investigated archaeomagnetically. The mudflow carries a magnetically hard remanence, the direction of which is significantly different from that of the present-day field, suggesting that the remanence is primary. The secular variation curve for Vesuvius agrees well with the Rome record and with other curves from Italy and London. A model for westward drift which accounts for certain features of secular variation in Europe over the past 200 yr requires a mean deceleration rate of $0.004 \text{ deg yr}^{-2}$. This is less than the current rate, suggesting that the deceleration rate was smaller 200 yr ago than it is today.

The historical lava-producing eruptions of Mt Vesuvius between AD 1631 and 1944 have recently been mapped in detail¹. Earlier events are poorly documented except the Plinian eruption of AD 79 which produced no significant lava. The period covered by the flows and the proximity of the volcano to Rome, where direct compass observations have been recorded since the sixteenth century, make an archaeomagnetic investigation particularly attractive because it is possible to compare the observatory with the archaeomagnetic records.

Eleven dated lava flows were sampled: where exposure permitted, flows were sampled at two or more sites (Table 1) located to obtain the greatest possible coverage of each flow. Five orientated 25-mm cores were collected at each site using routine techniques. Twenty orientated specimens of the mudflow which engulfed Herculaneum during the AD 79 eruption were collected from inside two adjacent rooms that had been filled completely by the mud. The finest silty material exposed was sampled by pressing small plastic boxes into prepared vertical surfaces.

Routine alternating field demagnetization indicated that the remanence at all sites was essentially single-component and stable. Angular shifts between demagnetization steps were small and random, and the mean median destructive field was close to 200 Oe. The natural remanent magnetization (NRM) and the cleaned flow mean directions are not significantly different (Table 1), but in all cases the precision is improved (average α_{95} is 2.7° and 1.9° respectively) and consequently the cleaned results are preferred.

The cleaned directions are plotted in Fig. 1a. The early part of the curve (excluding the point for AD 79) is indicated by a dashed line because this 330-yr span is defined by only two points. The general features of the curve are: a shift from shallower inclinations between the fourteenth and seventeenth centuries, and an incomplete clockwise elliptical loop in the subsequent period up to the present.

Observations during the past few centuries show that the curve of secular variation in Europe has been proceeding in a near-elliptical clockwise loop; Fig. 1b shows the curve for Rome. The shapes of the curves in Fig. 1a and b agree, bearing in mind that the 95% confidence limits on the Vesuvian directions are typically 1° or 2° . Inclinations at Vesuvius are expected to be $\sim 1^\circ$ shallower due to the latitude difference, but even after allowing for this, the Vesuvian curve remains 2° or 3° too shallow. A similar discrepancy was noted in the lavas of Mt Etna by Tanguy² who suggested that it was caused by magnetic refraction. Tanguy's results are replotted in Fig. 1c. The latitude difference requires that this curve be shifted $\sim 3^\circ$ to steeper

inclinations for comparison with the Vesuvian results, and when this is done the elliptical portions of the two curves show good agreement. There is, however, a significant difference in the early parts of the curves: Tanguy's curve seems to describe part of a counterclockwise loop with no indication of movement from shallower inclinations. Mt Etna is only 300 km south of Ischia and it seems unlikely that the difference is due to real variations in the non-dipole field. The discrepancy does depend to a large extent on the point for the lava flow of 1329. If this flow were wrongly identified (the incompleteness of early records makes this a possibility) the point may be disregarded, and this brings the two curves into better agreement over a longer interval. Tanguy expressed doubt about the reliability of the date.

It is interesting to compare the present observations with the record for London³ which displays a large shift from shallower inclinations between AD 1300 and 1600 followed by the well-documented clockwise elliptical loop. The corresponding movement in Italy is not so large, but identical angular displacements are not to be expected because the two localities are

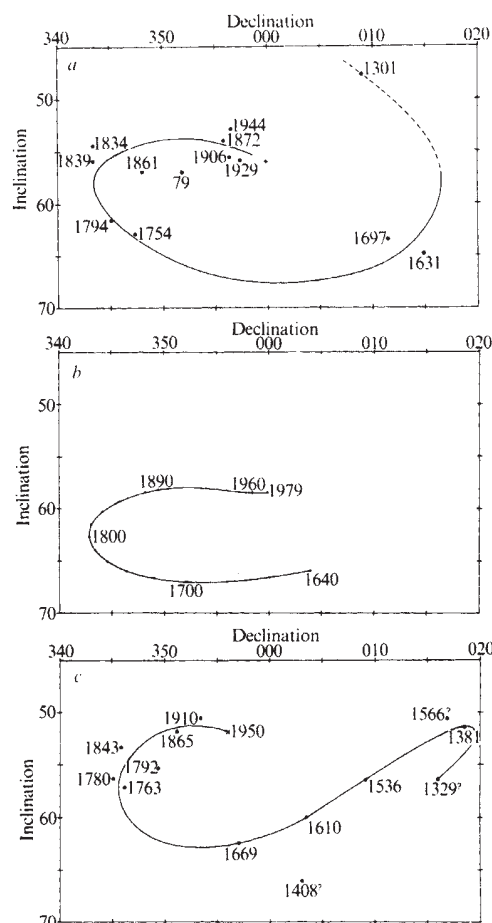


Fig. 1 a, The secular variation record for Mt Vesuvius. The dated points show the cleaned flow mean directions in Table 1. The present field direction is indicated by the cross. The direction for AD 1301 is from the lava del' Arso, Ischia⁶ which is only 40 km to the west of Vesuvius. The point for AD 79 is obtained from the mudflow at Herculaneum. b, The secular variation record for Rome derived from observatory records. 1979–60 supplied by F. Molina (personal communication); 1890–1640 taken from ref. 7. Bauer's reconstruction for the period 1640–1508 should be disregarded although the easterly declination is not in doubt. Bauer describes an observation of declination 005° for 1436, and there is a record of declination 007° for the eastern Mediterranean in the mid-sixteenth century⁸. c, The secular variation record for Mt Etna from Tanguy². A question mark by a point indicates that he doubted the date of the flow sampled. The point for 1950 is from ref. 9.

Table 1 Archaeomagnetic results from Vesuvius and from the lava del' Arso, Ischia*

Date of flow†	Site	<i>J</i>	Dec	Inc	α_{95}	<i>k</i>	<i>N</i>
1944	A	4.61	353.2	53.6	1.6	2,381	5
			353.0	53.6	1.8	1,884	
	B	4.05	359.7	50.7	1.1	4,871	5
			000.1	52.1	1.1	4,578	
		4.33	356.5	52.2	1.7	771	10
		356.6	52.9	1.7	833		
1929	A	4.27	357.1	56.7	1.4	2,988	5
			357.6	56.7	1.2	3,809	
	B	5.03	357.3	54.8	1.5	2,490	5
			357.3	55.2	1.5	2,740	
		4.65	357.2	55.7	1.1	2,103	10
		357.4	55.9	0.9	2,693		
1906		4.88	356.1	55.8	1.2	3,838	5
			356.5	55.6	1.1	4,842	
1872	A	4.47	355.8	56.1	4.2	271	5
			357.8	55.3	2.4	988	
	B	4.22	358.1	49.7	4.7	271	5
			354.0	52.6	1.9	1,633	
		4.35	357.0	52.9	3.3	209	10
		355.8	54.0	1.7	807		
1861		3.57	348.1	54.3	5.0	237	5
			348.2	57.0	3.1	593	
1839		3.53	343.3	55.7	2.7	819	5
			343.4	55.8	2.3	1,132	
1834	A	4.05	341.5	53.5	3.2	580	5
			340.9	53.1	3.0	645	
	B	3.82	347.3	54.7	2.6	861	5
			346.0	55.5	1.8	1,882	
		3.94	344.4	54.2	2.1	550	10
		343.4	54.4	1.9	653		
1794		3.98	345.7	60.4	1.7	1,928	5
			345.2	61.6	1.0	5,614	
1754	A	4.82	347.9	61.9	2.0	1,529	5
			348.3	62.5	2.1	1,335	
	B	6.02	347.2	61.5	3.3	530	5
			347.5	62.9	1.9	1,620	
	C	4.15	346.2	63.3	1.8	2,702	4
		346.2	63.3	1.7	2,930		
		5.00	347.2	62.1	1.2	1,022	14
			347.4	62.9	0.9	1,857	
1697	A	9.22	006.2	62.4	1.9	1,708	5
			006.2	62.6	1.7	2,017	
	B	6.29	018.7	63.5	2.4	1,000	5
			017.2	64.2	1.9	1,611	
		7.76	012.3	63.1	2.2	471	10
		011.5	63.5	1.9	618		
1631	A	3.14	020.2	57.3	7.1	118	5
			014.2	63.4	1.5	2,553	
	B	4.15	014.4	64.9	1.9	1,682	5
			015.6	66.2	3.1	627	
		3.65	017.7	61.2	4.0	147	10
		014.9	64.7	1.7	772		
1301*		3.2	010.6	47.3	2.1	209	24
			009.0	47.7	1.2	566	14
79‡		0.28	350.2	54.9	5.3	39	20
			351.9	57.0	2.2	212	

Entries in normal type refer to sites. Those in bold type give flow mean data. The first line of each entry (site or flow) refers to the NRM, the second to the partially demagnetized remanence. In cases where there is only one site per flow, site and flow means are the same. *J*, site or flow mean intensity of magnetization in 10^{-3} e.m.u. cm^{-3} ; Dec, declination; Inc, inclination; α_{95} , Fisherian 95% confidence cone about the mean; *k*, Fisherian precision parameter; *N*, number of individually orientated specimens used for the statistics. One core from a site in the flow of 1754 was misorientated and its direction rejected.

* Taken from ref. 6. † Dates are all AD and are taken from the map of Principe¹. Flows shown on the available topographical maps are not always reliably identified. ‡ Mudflow.

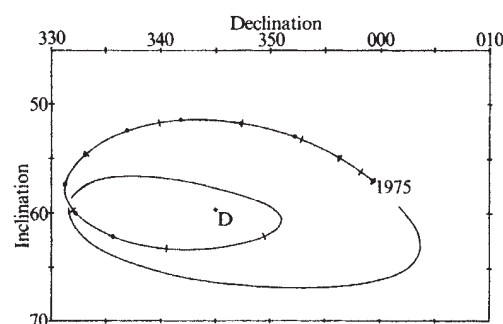


Fig. 2 Two models for the secular variation curve at Vesuvius, based on the IGRF75 representation of the main field. The dots show the predicted direction at 100-yr intervals from 1975, for a uniform drift rate of 0.1 deg yr^{-1} . The transverse bars give the direction at 25-yr intervals from 1975, for a deceleration rate of $0.004 \text{ deg yr}^{-2}$ to a velocity of 0.1 deg yr^{-1} in 1975. Point D indicates the dipole field direction.

far enough apart to be affected in different amounts by the same non-dipole anomalies.

It is not possible to extend the secular variation curve to include the point for Herculaneum because of the interval of 1,200 yr between this point and that for Ischia. The direction for Herculaneum, which lies within the recent ellipse, is not unreasonable, however. In the London record, the direction for AD 79 lies just outside the ellipse. This similarity in position, and the stability of the remanence, suggest that the mudflow did indeed record the ambient field direction at the time of emplacement. The mechanism of magnetization remains uninvestigated but it may be a shear-induced remanence⁴ acquired when the mud came to rest.

A theoretical secular variation curve for Vesuvius based on the 1975 International Geomagnetic Reference Field (IGRF75) is illustrated in Fig. 2. The direction of the dipole component of the main field, which is defined by the first three spherical harmonic coefficients, has remained effectively constant since 1829 whereas the dipole moment has decreased by 6%. In the model the dipole contribution is taken as fixed, and the non-dipole field, which is given by the higher-order coefficients, is driven westwards at the rate of 0.1 deg yr^{-1} . This rate is a reasonable estimate for 1975 and is taken from ref. 5. The secular variation curve for this model consists of a double clockwise loop about the dipole direction (Fig. 2). Although the whole curve is shifted to the west because of the orientation of the stationary dipole, the ranges of declination and inclination and the general elliptical shape are comparable with observation (Fig. 1). However, the time scale, which is determined by the rate of westward drift, is not suitable. For example, the time of maximum westward declination which was ~ 1810 occurs in the model ~ 1250 . In fact the model is in fair agreement with observation only in the present century. Better agreement over the past two centuries can be obtained if the westward drift is allowed to decelerate. A first-order calculation for a uniform deceleration which brings the time of maximum westward declination to ~ 1810 (Fig. 2) yields a mean deceleration of $0.004 \text{ deg yr}^{-2}$. This is about half the observed rate, averaged over the past three decades of 0.01 deg yr^{-2} (derived from Fig. 2 of ref. 5). The same calculation for London yields the same mean deceleration, which suggests that westward deceleration of the non-dipole field has been occurring on a continental scale in Europe over the past two centuries. The absence of a double loop in the record implies that the model breaks down after a few centuries, and this is in accordance with the recognized transient behaviour of non-dipole features.

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Stromatolites at ~3,500 Myr and a greenstone–granite unconformity in the Zimbabwean Archaean

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Two controversial areas of geological endeavour are the establishment of the antiquity of life and the tectonic setting of greenstone sequences. We record here the recent discoveries in the Fort Victoria greenstone belt of stromatolites in limestones assigned to ~3,500 Myr (minimum age) Sebakwian Group rocks of the Rhodesian Archaean Craton within Zimbabwe, and a nearby outcrop of a thin sedimentary formation, basal to a thick ~2,700 Myr volcanic pile, resting with definite unconformity on ~3,500 Myr Mushandike Granite.

Granite–greenstone terranes of different ages are now known to exist in the Rhodesian Craton^{1–3}. The oldest greenstone belt remnants constitute the Sebakwian Group dated at ~3,500 Myr minimum on the evidence from various granites and gneisses; the more extensive main greenstone belts comprise the Bulawayan and Shamvaian Groups (Table 1). An unconformity in the Bulawayan Group allows its subdivision into the Lower Greenstones (?~2,900 Myr) and the Upper Greenstones, which together with the overlying Shamvaian are ~2,700 Myr old. Two suites of late granite post-date greenstone belts. These are the largely tonalitic Sesombi Suite at ~2,700 Myr, and the

Table 1 Simplified stratigraphic table of the Zimbabwean Archaean

	Age (Myr)
Chilimanzi Suite (Adamellite)	~2,600
Sesombi Suite (Tonalite)	~2,700
Shamvaian Group	~2,700
Bulawayan Group { Upper Greenstones	~2,700
Lower Greenstones	?~2,900
Mushandike Granite	~3,500
Sebakwian Group	~3,500

dominantly adamellite Chilimanzi Suite at ~2,600 Myr. Archaean stromatolites in Zimbabwe have been recorded only from ~2,700 Myr Upper Greenstones⁴; further the only recorded and clearly demonstrable occurrence of greenstone belt rocks deposited unconformably on granitic basement is from the eastern margin of the Belingwe belt⁵.

The newly discovered stromatolites occur in limestones some 9 km east of Mushandike Dam wall, in the northern margin of the Fort Victoria greenstone belt, and within the Mushandike National Park (Fig. 1). These limestones are confined to the vicinity of the lake and to the east. They are part of a sedimentary unit that has its greatest development west of Mushandike Dam. This unit was originally regarded by Wilson⁶ as belonging to the Bulawayan succession. Later, as part of a craton-wide reassessment, Wilson² assigned the bulk of this sedimentary unit and some associated metavolcanic rocks to the ~3,500 Myr Sebakwian Group of the area. He was undecided, however, on the status of the sedimentary rocks east of the Dam because of the effects of later (?~2,700 Myr) tectonism.

Our recent fieldwork has indicated the presence of two greenstone belt successions in this area (Fig. 1)—one clearly younger, and one which we regard as older than the Mushandike Granite dated at $3,445 \pm 260$ Myr ($\lambda^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$; errors at 2σ)⁷, equating with the Upper Greenstones and Sebakwian Group respectively.

We propose to call the basal sediments of the Upper Greenstones the Gwenya formation, after Gwenya hill on the western flanks of which an 80-m thick sequence of ferruginous siltstones, with minor arkose and quartzite lenses, lies directly on the Mushandike Granite (Fig. 2). The sediments all dip at 50° SE and young to the south, away from the granite. The unconformity contact is essentially planar with minor undulatory features which may be wash-out channels. It is not faulted.

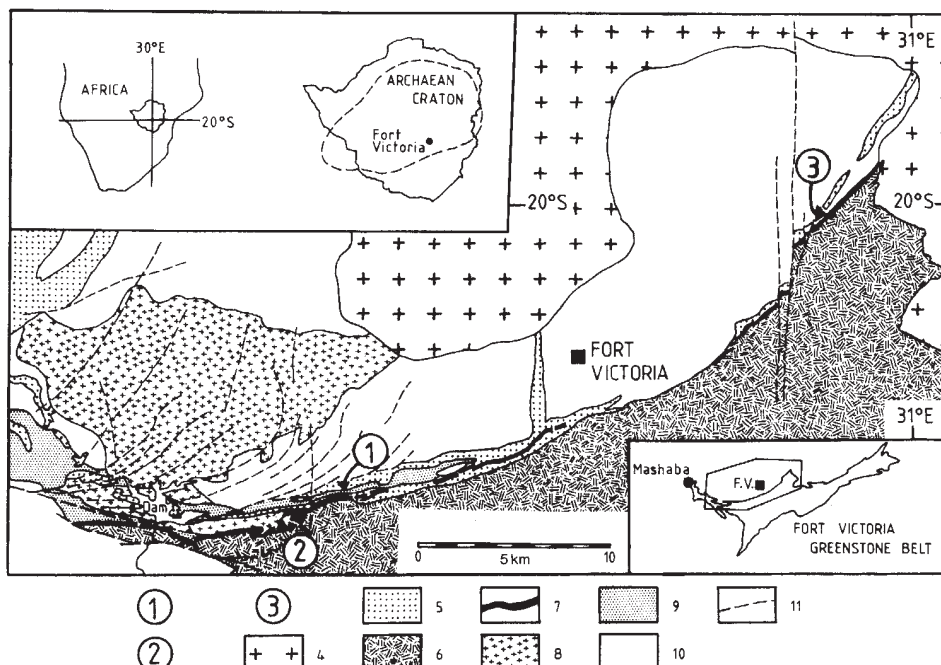


Fig. 1 Simplified geological map of the northern margin of the Fort Victoria greenstone belt. 1, Mushandike stromatolite locality; 2, Gwenya hill unconformity exposure; 3, Rockwood farm unconformity exposure; 4, Chilimanzi granite (~2,600 Myr); 5, Mashaba Igneous Complex; 6, Upper Greenstones volcanic rocks; 7, Gwenya formation (~2,700 Myr); 8, Mushandike Granite (~3,500 Myr); 9, Sebakwian Group; 10, various gneisses; 11, shear zones.

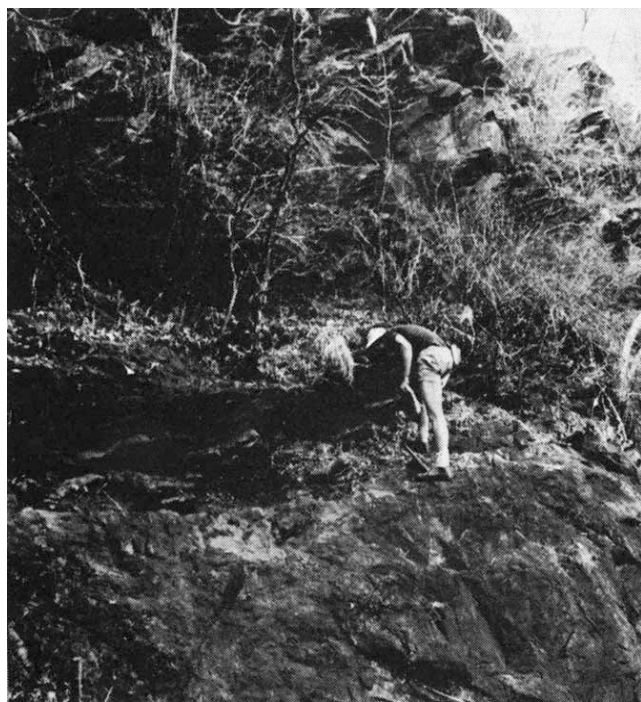


Fig. 2 Gwenya hill unconformity exposure. Left shoe straddles contact between weathered Mushandike Granite regolith and well-cleaved siltstones of Gwenya formation. A shallow depression in the centre of the picture forms a possible wash-out channel now filled with bedded siliceous siltstone.

West of the hill, the Gwenya formation is displayed by minor north-south faults, but seems traceable to south of the Mushandike Dam wall. Dips steepen westwards to 70° – 80° and as the wall is approached intense east-west shearing, slightly discordant to strike, has so disrupted the geology that we cannot establish field relationships between granite and sediments. However, Coward and Shackleton⁸ claim to have located an unconformity exposure, apparently from this region.

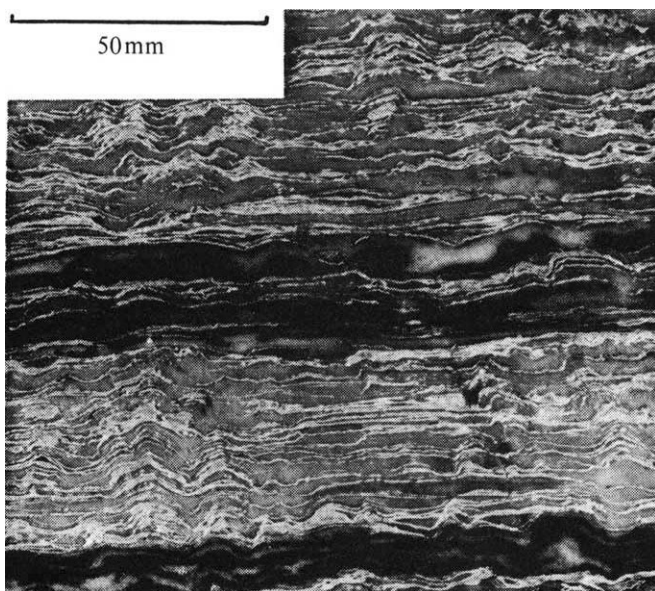


Fig. 3 Cut and etched surface of Mushandike stromatolitic limestone. Dark layers are of metasiltstone. Note preponderance of convex upward structures, moderate inheritance of domed laminae forming pseudocolumns in which successively younger laminae often develop a second order of curvature and thickening of laminae over the crests of flexures.

To the east of Gwenya hill, the sediments can be traced intermittently for some 40 km along the northern margin of the greenstone belt. For the most part the formation consists of ferruginous, argillaceous sediments, banded iron-formation and a few small limestone pods. For much of this sector of the northern margin, the Gwenya formation is bounded below, and in places above, by the peridotitic eastern arm of the intrusive Mashaba Igneous Complex⁶. Approximately 20-km north-east of Fort Victoria, the Upper Greenstones and Mashaba Igneous Complex are cut by ~2,600 Myr Chilimanzi granite. On the Rockwood farm road, 4 km south-west of this contact, the Gwenya formation sediments are poorly exposed in unconformable contact with the underlying gneisses.

The older sedimentary rocks, of which the limestones are an integral infolded part, are an eastwards continuation of the Mashaba Sebakwian Group. Unlike the Gwenya formation rocks, which together with the overlying volcanic rocks of the Upper Greenstones have suffered only low-grade metamorphism, these sediments show widespread evidence of a medium grade, contact metamorphic event. Para-amphibolites, containing cordierite, show stellate clusters of anthophyllite, up to 100 mm across, cutting an earlier tectonic fabric. Relict, but occasionally fresh, andalusite has developed in metapelites



Fig. 4 Outcrop of Mushandike stromatolites showing large oblate domes with high laminae inheritance characteristics. Lens cap is 5 cm in diameter.

adjacent to the limestones and in pelitic inclusions both within the Mushandike Granite and the surrounding gneisses⁶. When unaffected by the shearing (? ~2,700 Myr), these andalusites have a random orientation. We attribute the development of these minerals to the thermal effects of the Mushandike Granite intrusion.

The limestone has a complex mineral assemblage, but commonly all specimens studied from widely spaced localities along strike contain the following: calcite, clinocllore, talc, tremolite, zoisite, grossularite and calcic plagioclase, which is compatible with the medium grade of the other Sebakwian rocks.

The stromatolites occur in a limestone unit that crops out over a 7-km strike, and has an average 200-m outcrop width (Fig. 1). Centimetre-thick, intercalations of metasiltstone are common. The limestone is in places massive, but is mostly finely laminated on a millimetre/centimetre scale, although the body has been tightly folded so that much of the lamination may be transposed foliation.

The stromatolite locality has escaped this intense deformation hence the biogenic structures have been fortuitously preserved.

The exposure is small and consists of a few scattered outcrops over an area of some 20 m². In these the millimetre-thick laminae are distinct, and following Hofmann⁹ and Buick *et al.*¹⁰, they are stratiform and corrugate, with a preponderance of convex-upwards structures in close to open spacing between laterally linked domes that have moderate inheritance. These form pseudocolumns, some of which develop a second order of curvature having the appearance of incipient dendroid branching (Fig. 3). Some of the larger domes are oblate and are formed from laminae with high inheritance characteristics (Fig. 4). In thin section the limestone has undergone extensive recrystallization, making recognition of microfossil structures hazardous. The morphology of the laminae, which characteristically thicken over the crests of flexures, does not resemble the plicated structure of stylolites, nor is it explicable as either a deformational or sedimentary structure.

The maximum height of any pseudocolumn is 3 cm, with the total relief of any lamina varying between 2 and 10 mm. The angle of inclination of a column axis to bedding is generally 90°, but may lean at 60°–70°. Hemispheroid radii are commonly up to 1 cm, but can be as much as 4 cm. The vertical increase in radius of successive hemispheroids is usually <0.1 mm, whereas the upward displacement of the centre of successive domes is 2 mm giving a growth factor of 0.2.

The relationship of the morphology described to named stromatolitic groups is uncertain, but the forms bear affinity to *Stratifera* and/or *Irregularia*.

The Mushandike stromatolite discovery is one of the most significant yet in the search for early life forms. To our knowledge they are the best example of ~3,500 Myr biogenic structures known and add to the growing body of evidence^{11–14} that life was manifest as early as ~1,000 Myr after the formation of the Earth.

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Syn-sedimentary gravity slides (growth faults) in the Coal Measures of South Wales

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Growth faults are an important class of syn-sedimentary fault which have been widely recognized in sub-surface studies of hydrocarbon-bearing clastic basin successions^{1–4}. They are listric normal faults which are active for a limited period during sediment deposition and therefore affect a discrete sedimentary interval. Whilst active, the faults substantially influence sedimentation and a thicker, generally sand-dominated succession accumulates on the downthrown side which is consistently towards the basin. The faults are not directly related to basement tectonics, and instead are thought to originate principally by gravity sliding within the sediment pile⁵. Overpressured/undercompacted conditions in buried muds aid development of the faults by reducing the slope required to initiate sliding⁵, and in some cases associated mud diapirism also contributes by creating steeper slopes⁶. Despite their widespread recognition in sub-surface studies, relatively few examples have been described from exposed successions^{7–10}, and none from the economically important Upper Carboniferous Coal Measures of Europe. Here we describe examples of growth faults recently discovered in an opencast site of the Coal Measures in South Wales and comment on their possible significance.

The faults occur in the Middle Coal Measures (Westphalian B–C) which is the principal coal-bearing interval of the South Wales coalfield. They are exposed in the National Coal Board opencast site at Llanilid, north-west of Cardiff (NGR SS975813) in which the succession from the Five Foot-Gellideg Seam to the Bottom Abergorki Seam can be examined. This succession comprises 222 m of mudstones, siltstones and sandstones in which 14 workable coal seams occur. Growth faults have been detected at two levels in the upper part of this succession between the Gorllwyn and Bottom Abergorki Seams.

The lower set of faults affects an 8-m interval, and both underlying and overlying strata can be seen to be unaffected by the faulting (Fig. 1). The succession at this level comprises a series of small-scale coarsening-upwards sequences, two of

which are affected by the faulting. The sequences commence with black, plant-rich shales which contain horizonated sideritic ironstone nodules. This facies coarsens upwards into siltstones with occasional thin (1–2 cm) sharp-based beds of fine-grained sandstone. The sandstones increase in thickness and frequency upwards and grade into a fine- to medium-grained sandstone unit. These uppermost sandstones contain abundant plant root-lets and ironstone nodules, and have a light-grey leached and mottled appearance typical of Upper Carboniferous sandy palaeosols. A coal seam overlies the fault-affected interval and is abruptly overlain by mudstones at the base of the next sequence. These coarsening-upward sequences reflect the repeated infilling of shallow-water environments, initially by the deposition of fine-grained sediment from suspension and subsequently by sand-laden currents. Rapid infilling of the area culminated in soil formation, plant colonization and peat/coal accumulation. The sequences may have accumulated in the interdistributary bays of a delta¹¹, or in shallow lakes or barrier-sheltered lagoons as small-scale deltas prograded into the area^{12–15}. The lack of affiliated large-scale coarsening-upward sequences of delta front or beach-barrier origin at this stratigraphic level favours a lacustrine setting, perhaps analogous to the Atchafalaya area west of the present Mississippi delta¹².

Six faults are exposed within this interval in a horizontal distance of 165 m (Fig. 1). Each fault plane dips to the east and the strata are downthrown in this direction. The faults have a curved cross-sectional profile which is steep at the top (50°) and flattens progressively into a bedding plane fault in the shales at the base of the lower sequence and the underlying coal. The downthrown strata dip at angles up to 20° from the regional dip in the mid-depth region of the fault planes and terminate abruptly against the planes. The faults are thin zones only a few centimetres wide with minimal brecciation and only a weak foliation. In one example the distal portion of a fault steepens slightly from a bedding plane fault into a low angle thrust. Minor, westerly-dipping antithetic faults occur in the down-faulted mass and are typically steeper and straighter than the major faults. At the top of the faulted interval the dipping, faulted beds are truncated in the distal parts of each fault block and some of the material eroded from these sites appears to have been redeposited in depressions immediately adjacent to the fault on the downthrown side. The upper part of the fault-affected interval is overprinted by the leached palaeosol horizon at the top of the second sequence. These faults are clearly syn-sedimentary as both underlying and overlying strata are

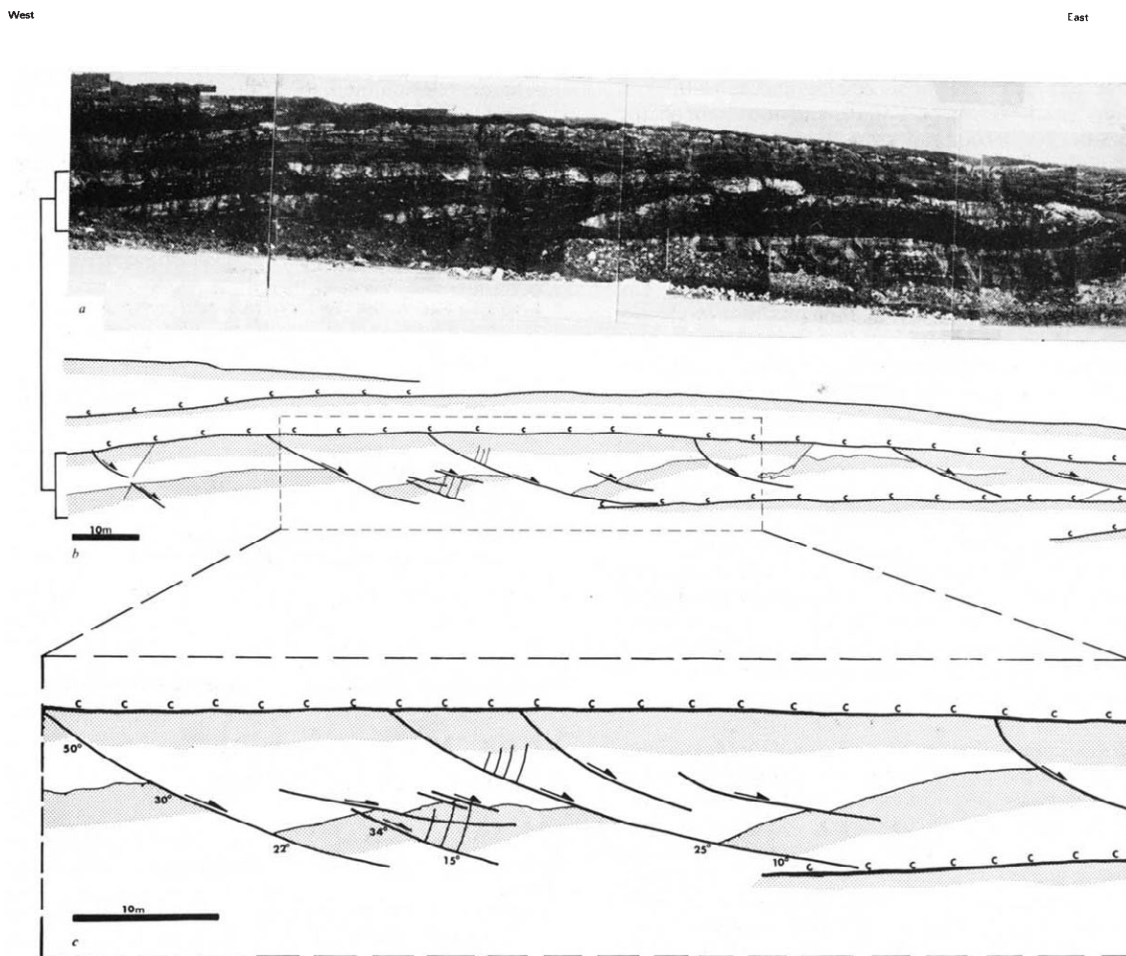


Fig. 1 *a*, Panorama of the lower set of growth faults in centre of photograph; *b*, line drawing of *a* showing the principal listric normal growth faults, occasional minor antithetic faults, the sandstones (stippled) and coal seams (*c*); note that both underlying and overlying strata are unaffected by the faults; *c*, detail of central part of *b* including dip angles of the faults.

unaffected, and the faulted strata were locally eroded before deposition of the next sequence. The major faults are listric normal faults reflecting easterly-directed gravity sliding. The orientation of the faults to the overall sediment transport direction is not known as the sedimentary structures have been obliterated by rootlets and palaeosol processes. The amount of deposition during faulting seems minimal, but is difficult to estimate in view of the small scale of the fault blocks and the extent of penecontemporaneous erosion in the distal parts of the fault blocks which has removed erosion evidence. However, it is clear that (? surface) relief created by the faulting was gradually obliterated by mass wasting processes possibly assisted by local wind-driven waves, resulting at least in re-sedimentation of this

material. Following the planation of the area a period of soil formation and plant colonization ensued, overprinting the levelled surface of the fault blocks.

The upper set of faults affects the succession immediately below the Bottom Abergorki Seam. At this level the succession comprises thinly bedded, current ripple-laminated sandstones with interlaminated siltstones and mudstones which are cut by a series of lenticular, channelized, fine- to medium-grained sandstones. These facies possibly represent the upper parts of river mouth bar systems cut by the fluvial channels which supplied them¹⁶, although research into this problem is still in progress. Three normal faults dipping generally eastwards are exposed in a horizontal distance of 45 m (Fig. 2). The principal fault has

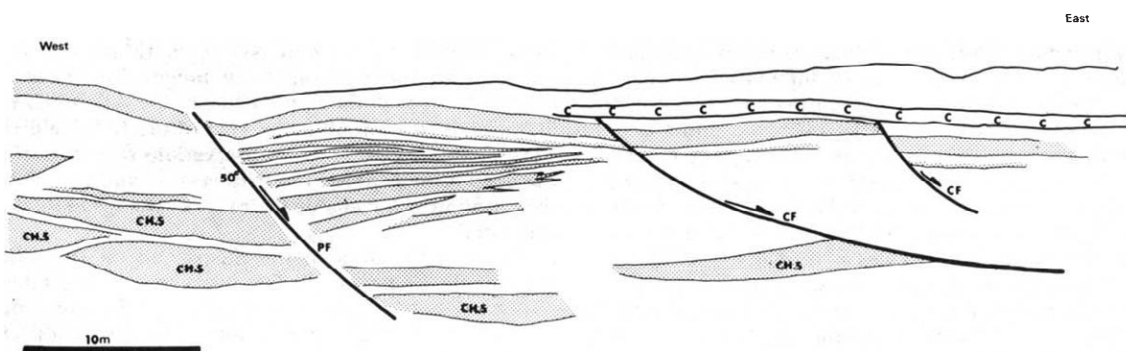


Fig. 2 The upper set of faults immediately below the Bottom Abergorki coal seam. The principal fault (PF) has only its upper part exposed, whereas the smaller scale, (?) crestal faults (CF) are completely exposed. Note the localized sandstone-dominated succession adjacent to the upper part of the principal fault which diminishes away from the fault. Sandstones are stippled. CH.S, channel sandstones; c, coal seam.

only its upper part exposed, dipping at 50° and flattening slightly with depth in the limits of exposure, suggesting a listric profile. This fault affects at least 18 m of succession and has a thicker, sandstone-dominated succession adjacent to the fault on the downthrown side. Numerous sandstone beds become thinner and finer grained when traced away from the fault plane, and eventually grade into siltstones and mudstones. These beds dip into the fault plane and are 'unconformably' overlain by the succeeding sandstone unit in the distal part of the fault block. Exposure of the fault plane permits the strike to be measured accurately at 072°/252°, indicating downthrow to the SSE. The preserved channel shape of numerous (inaccessible) sandstone units implies that the overall sediment transport direction was at a high angle to the strike of the faults. The localized sandstone-dominated facies on the downthrown side of the principal fault suggests that movements along this fault created a localized depression in the depositional area which became a prime site for the accumulation of sand-grade sediment. Differential sedimentation of this nature may have perpetuated the fault movements for some time causing the fault to grow until the locus of sedimentation shifted away from this area. On the downthrown side of the principal fault are two fully exposed, smaller scale faults with listric profiles. These faults parallel the principal fault and are downthrown slightly in the same direction, but do not exhibit a facies contrast between upthrown and downthrown sides. These smaller scale faults are clearly subordinate to the principal fault and are interpreted as minor, synthetic faults, possibly akin to the crestal faults of Evamy *et al.*⁴ in the growth-faulted Tertiary depocentre of the Niger delta.

The described faults are therefore syn-sedimentary, listric normal faults which are interpreted as growth faults and are considered to have been produced by gravity sliding directed generally east or south-southeast. Evidence for sedimentation during faulting is limited for the lower small-scale faults, but is clearer for the upper, larger scale faults. Formation of the faults may have involved overpressured/undercompacted conditions in the mudstones which reduced the slope required to initiate sliding. Previously it was felt that relatively deep burial was required to produce overpressured conditions, but *in-situ* measurements of pore pressures in delta plain and delta front muds of the Mississippi delta have recently detected overpressured conditions at very shallow depths below the sediment surface^{17,18}. In these conditions mass movement of sediment has occurred in the Mississippi delta on slopes as low as 0.14° (ref. 19), and Crans *et al.*⁵ consider that the slope required to initiate gravity sliding diminishes to <3°. Slopes towards the lower end of this range of values may have existed on the small- to moderate-scale deltas in which the faults occur. As in the Mississippi delta, excess pore-water pressures may have been supplemented by methane gas (as a bubble phase?) in the richly organic muds of the Coal Measures.

The recognition of growth faults in the Coal Measures has considerable potential significance because if they prove to be common, particularly on a larger scale, they may influence the location, lateral extent, frequency, thickness and disposition of coal seams. Although the described faults are small by comparison with many of the sub-surface examples described from hydrocarbon provinces, they clearly influenced sedimentation and the resultant facies patterns (particularly the upper, larger scale example). The scale of the observed faults is in proportion with the scale of the exposures and larger scale examples may be recognizable in seam-correlated cross-sections through coalfields. A series of larger scale listric normal faults known collectively as the Jubilee Slide has been described in sub-surface studies of the South Wales coal basin in the Pontypridd area²⁰. This structure comprises numerous slightly arcuate listric normal faults arranged *en echelon* in a fault zone traceable for 20 km along strike. The fault system affects the Lower, Middle and Upper Coal Measures and has a maximum throw of 90 m. The structure pre-dates later tectonic structures, but cannot be regarded as a syn-sedimentary growth fault as there is neither evidence that the faults influenced sedimentation, nor

that they are overlain by beds not affected by the faulting. In view of the growth faults described herein the Jubilee Slide should be re-examined to determine whether or not it is a large-scale syn-sedimentary growth fault.

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Visual observations of the Red Sea hot brines

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The research vessel Akademik Kurchatov has investigated the Red Sea rift zone with the aid of a manned submersible Pisces XI in the winter of 1979–80, as the value of such investigations is now well established^{1,2}. Our research involved a comprehensive programme of hydrophysical studies in deeps containing thermal brines of the sea axial rift—Atlantis II, Discovery and Valdivia (see Fig. 1). Measurements were made with standard hydrographic stations, the AIST temperature and salinity meter with specially selected temperature and electric conductivity scales on board a towed vehicle Zvuk-4m, and Pisces XI. The present data on water stratification in the Atlantis II, Chain and Valdivia deeps have in general confirmed previously known data^{3–9} but have also revealed some new facts. For example, we have recorded an increase of temperature of the hot brine of up to 62 °C in the southwestern basin of the Atlantis II deep since studies aboard the R/V Sonne in 1977. Using data from this expedition, Hartmann⁹ gives a curve of temperature growth in the lower brine constructed from the data of different expeditions since 1965. A multilayer temperature structure of the upper brine and horizontal inhomogeneities have also been found^{8–10}. Steps in the upper brine of the Atlantis II deep were first described^{6,9} from the surveys of the R/V Valdivia (1971–72) and Sonne (1977). Our observations reveal stratification of the deep brine (Table 1) in temperature and electrical conductivity (salinity) and enable their rate of change of thickness to be estimated¹⁰.

Water in the area of the deeps and on the relief saddle between the Chain and the Discovery deeps was sampled using a 15-l water bottle designed to function at 5–10 m depths above the sea floor. The samples were analysed hydrochemically (Table 2) as well as in a spectrohydronephelometer which measured spectral transparency curves.

A comparison of these curves for the bottom water shows that in the Atlantis II deep a sharp decrease in transparency is

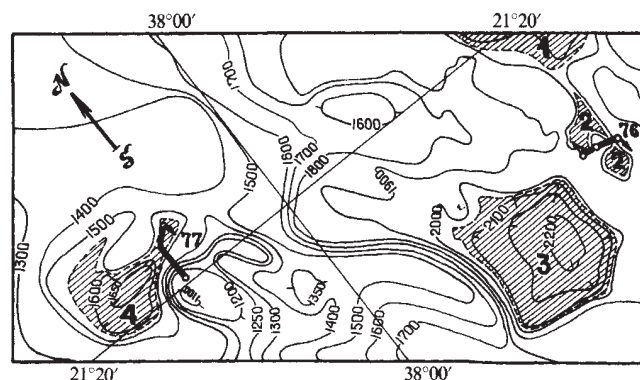


Fig. 1 A scheme of the "Pisces XI" dives into the Chain (76) and Valdivia (77) deeps: 1, Atlantis II deep; 2, Chain deep; 3, Discovery deep; 4, Valdivia deep.

observed at ~ 11 m above the bottom (for $\lambda_{\text{eff}} = 546$ nm): a 10-m thick turbid bottom layer seems to exist. The transparency curve of the Valdivia deep resembles the curve from the Atlantis II deep; however, in the Discovery deep the bottom water differs appreciably. On the saddle between the Chain and the Discovery deeps the optical properties of the water are intermediate between those of the Valdivia and the Discovery deeps. Note that the Discovery and the Atlantis II waters differ considerably in their chemical composition (Table 2). Our analyses indicate that the nature of the brines changes within the deeps.

Taking into consideration the large amount of data available for this region, our expedition aimed to verify the hypothesis of a common source of thermal water in the Atlantis II, Chain and Discovery deeps^{4,7,8} and to investigate the Valdivia deep for which only scanty data have been published⁵.

The hypothesis of the formation of high-salinity thermal water in the Chain and the Discovery deeps assumes an overflow

Table 1 Thermohaline characteristics of water in brine steps within the Atlantis II deep

No. of steps	Thickness (m)	Temperature (°C)	Salinity (%)
1	4	40.9	67.6
2	8	47.1	113.6
3	16	51.5	144.3
4	10	52.2	145.1
5	From 2,049m depth to the bottom	62.3	275.0

of this water through bottom depressions from the southwestern basin of the Atlantis II deep.

The Pisces XI submersible was equipped with hydrophysical sensors and a dive was made to the supposed place of contact of thermal waters between the Chain and the Discovery deeps (see Fig. 1, route 76). Meridional towing of the Zvuk-4m vehicle across the saddle had already yielded 14 photographs of the saddle bottom (see, for example, Fig. 2). The photographs show a highly dissected topography with rock outcrops and numerous fragments of apparently basaltic rocks powdered with sediment often white and showing ripple marks in some areas. Figure 2 *a*, *b* indicates a complicated bottom topography within the saddle. Steep step-like scarps of a 3–4 m amplitude have been fixed (Fig. 2*a*). In the vertical walls, bedrock crops out, flat areas are powdered with loose sedimentary material. Judging by the bedrock fragments, we have observed, not effusive formations encountered in the axial rift zone, but carbonate strata (marls) or iron–manganese consolidated sediments the fragments of which were found in the submersible's ski (dive 76). A tectonic origin of the scarps is most probable. Ripples are seen on the scarp to the right of the rock in Fig. 2*a*, on the left-hand part of the photograph in Fig. 2*b* and on the whole of Fig. 2*c* which reveals mysterious decimetre-scale ripples (possibly, on the brine surface).

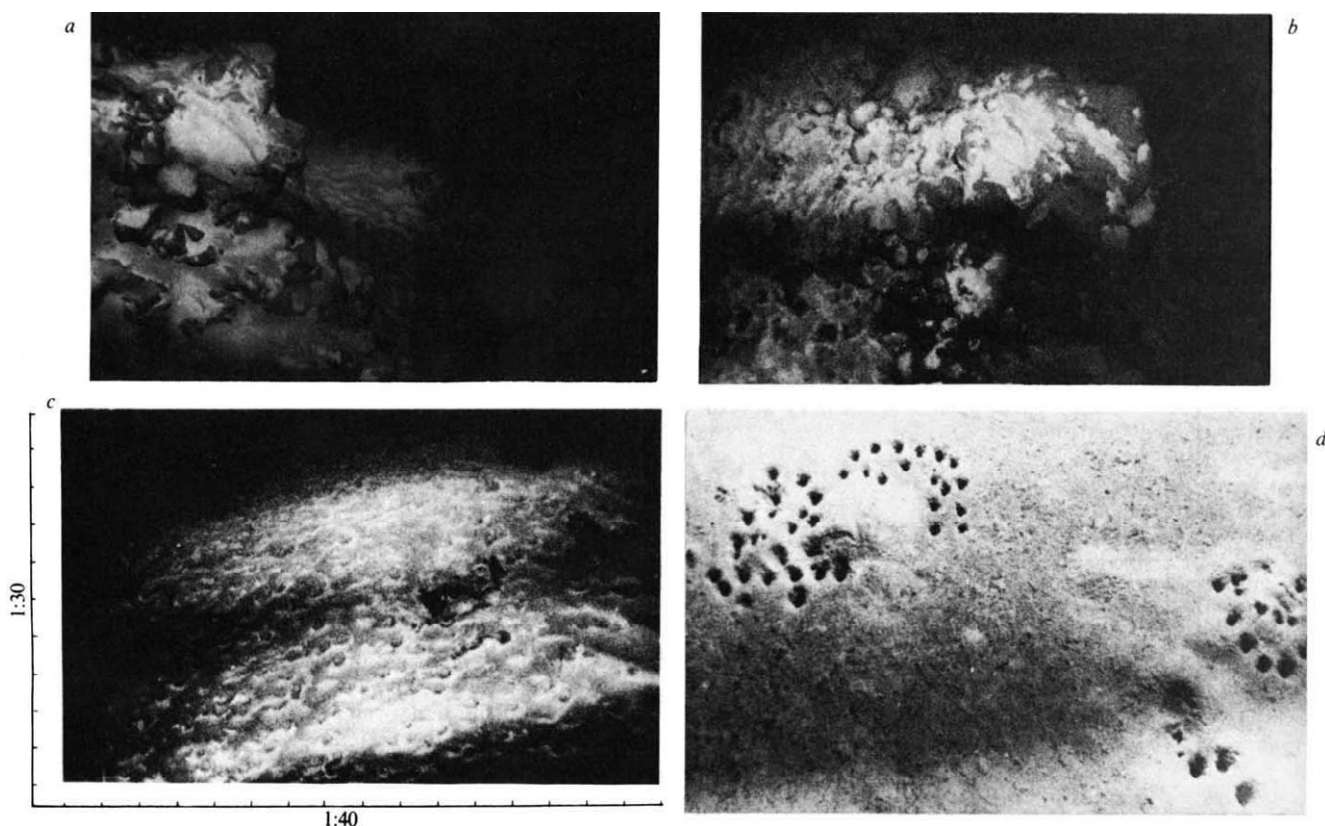


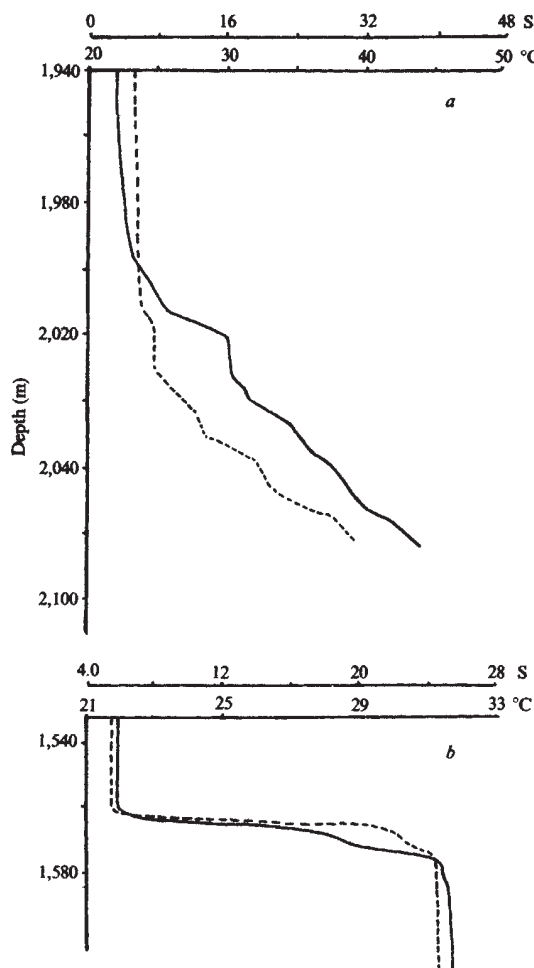
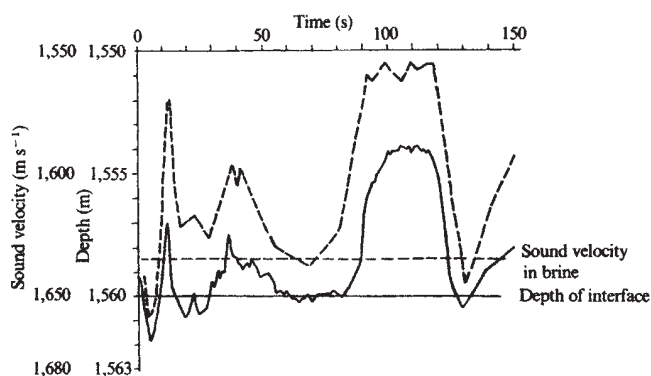
Fig. 2 Bottom and brine surface photographs made in the diving area with the aid of the towed vehicle Zvuk-4m: *a*, bottom area covered with ripples (to the right of the rock); *b*, bottom area with ripples (in the left-hand part of the photograph); *c*, decimetre-scale ripples (possibly, on the brine surface); *d*, a group of bottom holes.

Table 2 Hydrochemical characteristics of deep brines and surface water in the Red Sea

	Brine level (m)	Transitional layer (m)	Sampling depth (m)	Temperature (°C)	Salinity (% conductometric, at bulk dilution)	Oxygen (O ₂ ml per l)	Si ($\mu\text{g atom l}^{-1}$)	Disposable CO ₂ (ml per l)	Total CO ₂ (ml per l H ₂ O)
Atlantis II									
Sample 1	2,028	1,975	2,127	62	321	0	800	5.6	44
Sample 2	2,056	2,052	2,070	62	322	0	—	5.8	43
Discovery	2,060	1,995	2,118	44	315	0.2	150	0.21	9.2
Valdivia	1,565	1,560	1,657	32	286	0	140	2.5	83
Red Sea	—	—	0	27	38.9	4.5	11	0.21	51

The Pisces XI dive (76) onto the Chain-Discovery saddle on 12 February 1980 showed that the water changed its transparent-blue colour to dull yellow (due to a high concentration of suspended matter in the upper part of the sharp pycnocline layer) at a depth of 1,995 m, as the bottom was approached. The water temperature increased from 21 to 25 °C and the sound velocity from 1,560 to 1,572 m s⁻¹. The sea floor at a depth of 2,005 m was covered with light-yellow sediment with easily turbidable black coating, straight parallel sand bars 1.5–2 m apart and with separate white spots, decimetres in diameter, and centimetre-high knolls (possibly, evaporite outcrops). Although there were no traces of life on the bottom a dead fish found there may be indicative of the conditions preserving organic matter, that is the absence of oxygen and bacteria.

The submersible moved towards the Discovery deep, just above the apparent bottom which looked to the observers like mud covered with waves. However, in their attempts at touching-down on this bottom the observers made sure that they saw (and the echosounder of the Pisces recorded) the brine surface.

**Fig. 3** Curves showing vertical distribution of temperature (solid line) and conductivity (dashed line) in the thermal brine layer of the Chain (a) and Valdivia (b) deeps.**Fig. 4** Graphic recordings of sound velocity and depth during the Pisces XI manoeuvres in its dives into the Valdivia deep (a sample of the recordings of the submersible's sensors during 150 s).

Going deeper into the brine the water became sharply turbid, a streaming yellowish haze could be seen through the portholes, overboard temperature increased to 33 °C and sound velocity to 1,625 m s⁻¹ (the Chain deep). At the maximum depth of 2,030 m the buoyancy force of the pycnocline balanced the forces of the thrusters and stopped the descent of the submersible.

The analysis of the hydrophysical and echosounder recordings made during the crossing of the saddle shows no evidence of brine overflows from the Chain to the Discovery deeps. Contact between the deeps takes place, evidently, only at the depth of the transition layer between the normal Red Sea deep waters and the brine layer (1,980–2,010 m layer, Fig. 3a).

Hydrophysical studies of the Valdivia deep (Fig. 1, route 77) were also undertaken. Preliminary standard hydrographic casts and soundings with the AIST had revealed a sharp pycnocline with a very thin transition layer (Fig. 3b). During the dive into the Valdivia deep, on 14 February, 1980 the submersible reached the bottom at a depth of brine of 1,530 m east of the deep. On setting course for the west, at a depth of 1,555 m the Pisces entered the transition layer which was immediately noted by the observers as a streaming viscous haze. A pycnocline layer as a turbid surface covered with ripples was clearly seen 3–5 m below the submersible. As in the dive to the saddle described above, no penetration through the pycnocline was possible, and after attempting to go deeper with the aid of its thrusters the submersible headed eastwards above the brine surface. The manoeuvres of the submersible during its attempts to go deeper into the brine are shown in Fig. 4 together with the results of continuous sound velocity measurements in the brine (a sample taken over 2.5 min is presented).

Moving above the brine surface the submersible reached its underwater 'shore' covered with yellowish mud and descended at 30–40° into the underwater salt 'lake'. Against the background of the slope the brine level was distinct, resembling a whitish mist surface, and the observers noted underwater waves at a depth of 1,560 m (internal waves on the brine surface which was disturbed by the Pisces thrusters) which were rolling on the shore of the salt lake and then were rolling down in streams.

Furthermore the Pisces started ascending along the bottom rise slope covered with sediments with light-yellow and brown

crests of thin-layered sedimentary rocks usually inclined towards the slope and covered with carbonate crusts with a dark yet thin manganese 'tarnish' on the surface. The inclined bedding of sedimentary rocks is most likely to be due to slide phenomena on the salt-bearing slopes of the Red Sea main trough.

According to M. S. Barash (personal communication) the samples taken during the dives from laminated sedimentary rocks contain abundant well-preserved late Quaternary planktonic foraminifers (*Orbulina universa* d'Orb; *Globigerinita glutinata* Egger; *Globigerinoides sacculifer* Brady; *Globigerinoides ruber* d'Orb; *Globigerinella aquilateralis* Brady; *Globigerina rubescens* Hofker; *Globigerinoides tenellus* Parker).

Ushakova studied the same samples and determined Pleistocene (*Emiliania huxleyi* Lohman; *Unibulicosphaera mirabilis* Lohman; *Geophyroacapsa oceanica* Kamptner; *Coccolithus atlanticus* Cohen) redeposited Cretaceous (*Lucianorh abdu cayeuxi* Deflandre; *Microrhabdulus decoratus* Deflandre; *Lithraphidites quadratus* Bramlette, Riedel) and Palaeogene calcareous nannoplankton (*Helicopontosphaera intermedia* Martini, Hay, Mohler; *Discoaster multiradiatus* Bramlette, Riedel; *Pseudozygrrhabdulus latus* Hag; *Marcalius inversus* Deflandre, Bramlette, Martini).

The carbonate crusts covering thin-laminated sedimentary rocks are identical in composition to those studied in detail in the Red Sea rift zone at 18°N where their formation is associated

with the climate cooling stages in Pleistocene. These crusts are most likely to be dated as Würmian. Their origin could be accounted for by a partial bridging of the Bab-el-Mandeb strait due to a general lowering of the World Ocean level during Würmian glaciation. Several metres above the lake level, separate pink shrimps and numerous groups of holes (1–3 cm in diameter and ~1 dm apart) were noted in the sedimentary cover, the latter being found almost everywhere on the Red Sea bottom (see, for example, Fig. 2d). No such groups of holes were encountered in the vicinity of the brine surface or on the bottoms of the thermal deeps. This may be evidence for their biological origin.

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Atlantic-type carbonate stratigraphy in the late Miocene Pacific

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In previous attempts to correlate fluctuations of climate with the carbonate content of deep-sea sediments, using the ratio $^{18}\text{O}/^{16}\text{O}$ as an indicator of palaeotemperature, conflicting patterns have emerged between Pacific and Atlantic deep-sea cores. Although there is controversy about the relative importance of the primary productivity of calcareous microorganisms^{1–3} and their dissolution at depth^{4–10}, in Pacific Pleistocene cores high-carbonate sediments correlate with glacial episodes and low-carbonate sediments with interglacials^{1–4,7–10}. In Atlantic Pleistocene cores, on the other hand, the correlation seems to be reversed^{4,5,7,10–12}. We report here that a late Miocene (6.14–6.53 Myr) deep-sea sediment core from the eastern equatorial Pacific (DSDP Site 158) shows a negative correlation between carbonate content and benthic oxygen isotopes which is similar to that found in Quaternary records of the Atlantic rather than the Pacific. The explanation may be that Atlantic and Pacific waters differed in HCO_3^- concentration at depth as they do today, and that in the late Miocene, Atlantic water flowed over the submarine sill at the Isthmus of Panama and at least periodically, reached the location of Site 158.

Stable isotope and carbonate analyses were performed on 44 sediment samples from DSDP Site 158 (Cocos Ridge, eastern equatorial Pacific Ocean, 06°37.36'N, 85°14.16'W, 1,953 m water depth). Samples were taken from cores 15 and 16 at intervals of 20 cm across the late Miocene ^{13}C depletion in benthic foraminifera^{13,14}, and thus, our samples span an interval of 390 kyr between ~6.14 and 6.53 Myr, based on the estimated age of 6.3 Myr for the carbon shift¹⁵. The sample spacing represents an interval of ~10 kyr between samples, based on

the estimated sedimentation rate of 2.0 cm kyr⁻¹ for the late Miocene¹⁶. Stable carbon and oxygen isotopic ratios of the benthic foraminifera¹⁴ *Globocassidulina subglobosa*, and benthic/planktonic (B/P) foraminiferal ratios were determined for each sample. Carbonate percentages for each sample were determined by the revised carbonate bomb techniques¹⁷.

Our results for Site 158 show that the highest percentages of carbonate correlate with lighter benthic oxygen isotopic ratios (Fig. 1a), indicating warmer palaeotemperatures and/or less continental ice volume. The maximum correlation occurs with an offset of one sample interval, suggesting that changes in the carbonate content lead changes in the oxygen isotopic content by ~10 kyr. This conflicts with previous studies of Pleistocene equatorial Pacific cores^{18–21} which estimated that carbonate oscillations lagged behind oxygen isotope variations by 5–10 kyr. The pattern of Site 158 is that of an Atlantic-type carbonate stratigraphy (high-carbonate interglacials); although the maximum magnitude of the correlation coefficient (0.42 at one sample lag) is low, it is of approximately equal magnitude with the maximum oxygen isotopic-carbonate correlations of Pleistocene cores V19–29 ($r = 0.52$) and V28–238 ($r = 0.35$) from the equatorial Pacific²¹, despite the fact that cores V19–29 and V28–238 show the opposite Pacific-type pattern.

Our data also show that higher B/P values correlate with lower carbonate percentages (Fig. 1b). The maximum correlation coefficient ($r = 0.54$) occurs at one sample lag, indicating that changes in carbonate content lead corresponding changes in B/P ratio by ~10 kyr, and that B/P ratio and oxygen isotope records are in phase. Because dissolution preferentially removes planktic foraminifera, higher B/P ratios indicate increased dissolution. The lag between these two indicators of dissolution is perplexing. Total carbonate content (both coccoliths and foraminifera) may respond more rapidly to changes in bottom water chemistry than do changes in foraminiferal faunas alone. The correlation of B/P ratios with per cent carbonate suggests that in the late Miocene (6.14–6.53 Myr) samples from DSDP Site 158, carbonate percentage is controlled primarily by dissolution. Furthermore, it indicates that times of greater $\delta^{18}\text{O}$ ('glacials') are times of greater carbonate dissolution, the pattern found in Pleistocene Atlantic deep-sea cores^{4,5,7,10–12}.

Spectral analysis of such short time series, especially those for which the time-spacing of samples is only approximate, cannot

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be expected to give very accurate estimates of the variance distribution as a function of frequency; however, such an analysis can give a general idea of how the variance is partitioned between the low and high frequency parts of the spectrum. More important, cross-spectral analysis^{21,22} of the carbonate and oxygen isotope paired time-series can provide a useful indication of the frequencies over which the two records are most highly correlated. The age model for our spectral analyses is based on the shift in benthic ^{13}C isotopes at 136.50-m depth in this site, which has been dated¹⁵ at 6.3 Myr, and the estimated sedimentation rate¹⁶ of 2.0 cm kyr^{-1} .

The oxygen isotope spectrum shows broad spectral peaks centred at frequencies corresponding to periods of 167 kyr, 53 kyr, and 22.7 kyr (Fig. 2). The carbonate spectrum shows broad peaks at 235 kyr and 29.4 kyr, with only a shoulder in the spectrum near the frequency corresponding to a period of ~60 kyr. These results agree with a study of the carbonate record²³ from the entire late Miocene of Site 158 which showed that most of the variance in the record is associated with periods of oscillation longer than 150 kyr. The presence of spectral peaks in both records near the periods associated with the changes in obliquity and precession of the Earth's orbit²⁴ may be only fortuitous.

A high degree of correlation (coherency) occurs between the oxygen isotopic and the carbonate signals of Site 158 at low frequencies (periods longer than 111 kyr, with a coherency peak

at a period of 182 kyr). A second region of high coherency is centred about a period of 30 kyr. The coherence between the two curves at long periods of oscillation may be due to matching a few peaks and valleys in a relatively short section of the carbonate record. However, the length of record (~390 kyr) is comparable with many of those which have been used to establish the relationship between carbonate and isotope stratigraphies in the Pleistocene¹⁸⁻²¹. Moreover, the coherency between the oxygen isotopic record and the carbonate record even at higher frequencies lends credence to the proposed relationship between the two records.

The discovery of an 'Atlantic-type' carbonate stratigraphy in the late Miocene eastern equatorial Pacific Ocean and the eventual development of the 'Pacific-type' stratigraphy may be related to the tectonic evolution of the Isthmus of Panama. The carbonate variations seen in the late Miocene at Site 158 are primarily due to dissolution, as shown by the B/P foraminifera ratios, with less dissolution occurring during the warmer (isotopically lighter) intervals. This pattern may have been caused by the exchange of water between the Atlantic and Pacific through the open seaway of Panama. Thus, before the middle Pliocene closure of the Isthmus of Panama^{25,26}, periods of warming (lower $\delta^{18}\text{O}$) may have been associated with increased flow of Atlantic waters across the Panama Seaway and into the Pacific basin. This water would be less corrosive to calcite because it would be younger and contain less dissolved CO_2 than

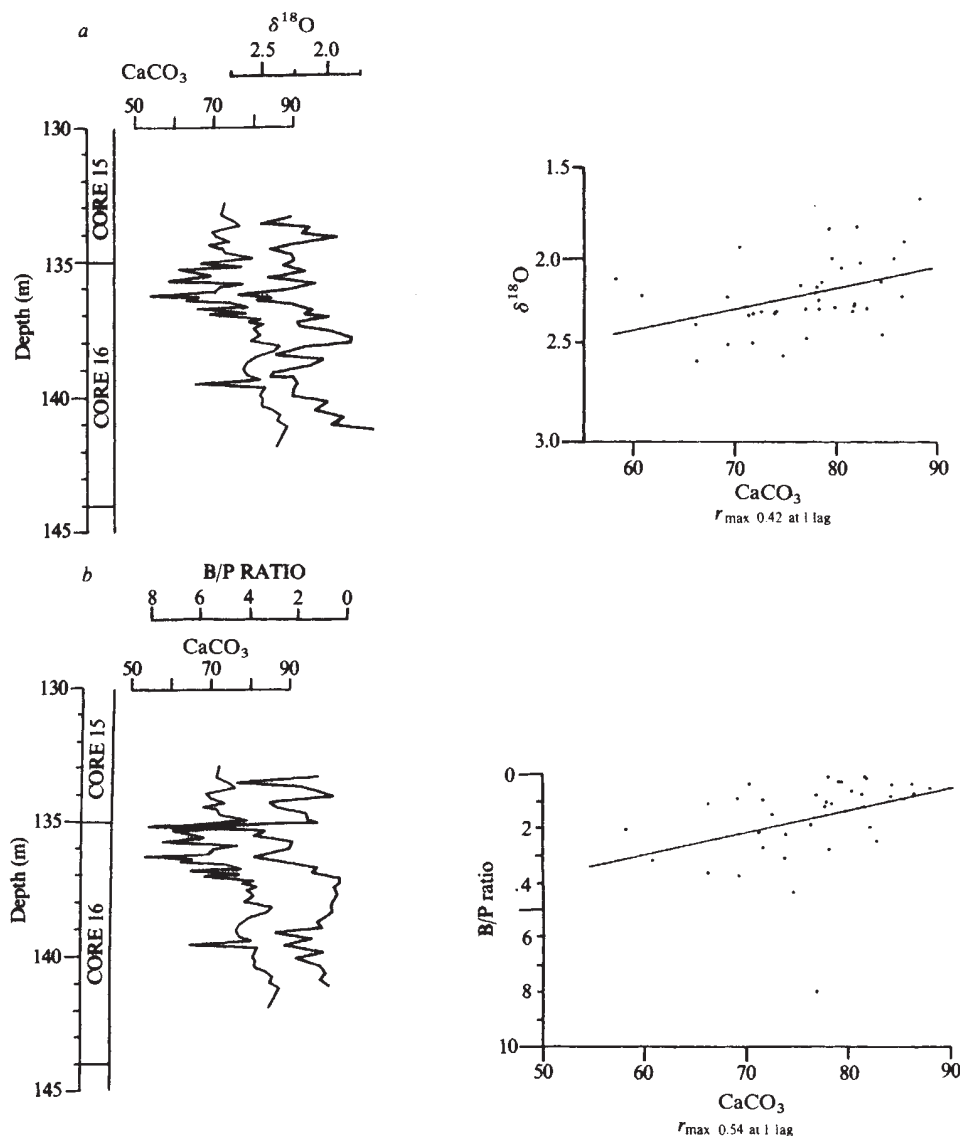


Fig. 1 a, Carbonate percentages and stable oxygen isotopic ratios of the benthic foraminifera *Globocassidulina subglobosa* (relative to the B-1 powder standard) for 44 paired samples in DSDP Site 158. Per cent carbonate and $\delta^{18}\text{O}$ are negatively correlated, implying that there are high-carbonate warm intervals (an 'Atlantic-type' carbonate stratigraphy) in this sediment from the late Miocene eastern equatorial Pacific. The y-axis ($\delta^{18}\text{O}$) has been inverted in this figure to make the Atlantic-type correlation more obvious. The maximum correlation coefficient ($r = 0.42$) occurs at one sample lag, indicating that changes in benthic oxygen isotopic content lag behind changes in carbonate percentage by ~10 kyr. b, Carbonate percentages and B/P foraminiferal ratios for DSDP Site 158 samples. Per cent carbonate and B/P ratios are negatively correlated, implying that variations in carbonate percentage are primarily due to dissolution, as shown by high B/P ratios correlating to low carbonate percentages. Maximum correlation coefficient ($r = 0.54$) occurs at one sample lag, indicating that changes in the B/P ratio lag behind corresponding changes in carbonate percentage by ~10 kyr.

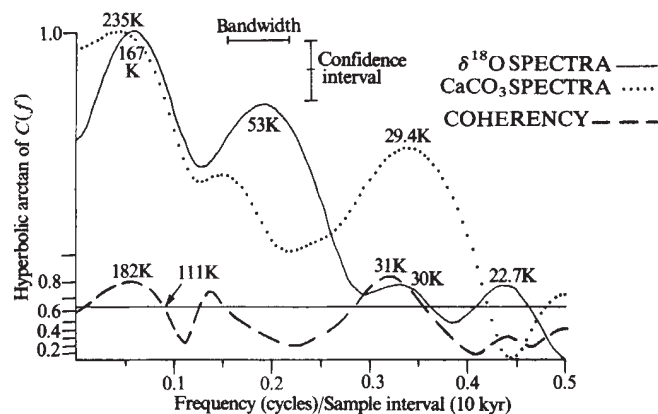


Fig. 2 Spectral analysis of stable oxygen isotopic ratios of *Globocassidulina subglobosa* and carbonate percentage, and coherency between these two signals. Horizontal axis is cycles per 10 kyr. Log spectra of both time series are plotted on the vertical axis as power (equal to mean variance squared) versus frequency. Spectral estimates for $\delta^{18}\text{O}$ and CaCO_3 have been scaled by a constant, so that the area under each curve equals one. Vertical axis for coherency (hyperbolic arctangent of coherency for each frequency) was chosen so that the confidence interval is constant for all frequency bands.

the Pacific waters. During cool intervals, Pacific water masses (which have been away from the surface longer and thus are higher in dissolved CO_2 and more corrosive to calcite) could have replaced those derived from the Atlantic. The exact mechanism which might drive this oscillation in the source of deep waters for the eastern tropical Pacific is uncertain, but it may be associated with differing rates of production of deep waters in the Northern and Southern Hemispheres.

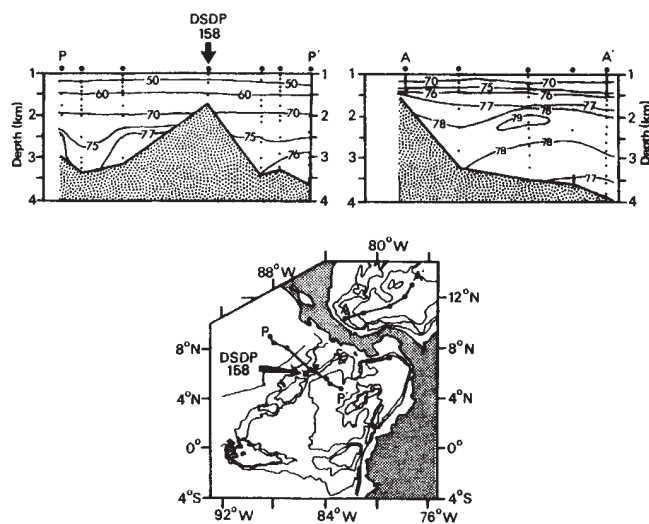


Fig. 3 Modern hydrographical sections for the Colombian Basin and the eastern equatorial Pacific. Density contours are plotted as values of $(\sigma_\theta - 27) \times 100$; a σ_θ value of 27.70 would be represented by 70. The estimated late Miocene palaeodepth of Site 158 is 1,700–1,800 m (ref. 31). Assuming that late Miocene density stratification of these basins was similar to the modern situation, then water masses passing over a minimum sill depth of 1,000–1,200 m would sink to the depth of Site 158.

At present, surface waters on opposite sides of the Panama Isthmus have similar surface temperatures (26–28 °C), but Atlantic waters have higher salinities. Flow of warm, saline Caribbean water from the Atlantic North Equatorial Current into the eastern equatorial Pacific has been proposed as a contributing factor to accumulation of Pliocene carbonate-rich sediments in the equatorial Pacific²⁷. Also, passage of shallow Caribbean waters across Panama in the Pliocene has been implied as the mechanism for migration of a shallow-water benthic foraminiferan (*Amphistegina gibbosa*), normally restricted to the Atlantic–Caribbean province, into sediments off the California Continental Borderland²⁸. Examination of modern hydrographic data^{29,30} from the western Colombian Basin and the eastern equatorial Pacific Ocean (Fig. 3) indicates that Atlantic water masses passing across the Panama sill would sink to greater depths in the eastern Pacific, due to high densities found at shallower depths in the Atlantic. (Additional hydrographic data for the Colombian Basin and the eastern equatorial Pacific was made available by the National Oceanographic Data Center.) Thus, flow of Atlantic waters westward into the Pacific is theoretically possible, with circulation being driven by sinking of Atlantic water masses along isopycnal surfaces to greater depths in the Pacific.

Based on an estimated back-tracked depth of 1,700–1,800 m for Site 158 during the late Miocene³¹, and assuming that density stratification of late Miocene ocean basins was similar to the modern situation, we estimate that the minimum depth for the late Miocene Panama sill was 1,000–1,200 m. This sill depth would allow Atlantic intermediate water masses to be transported through the Central American seaway. In the eastern Pacific, the Atlantic water masses could sink to the estimated depth of Site 158, causing the reduced carbonate dissolution at this site and producing the ‘Atlantic-type’ carbonate stratigraphy. If this proposed hypothesis is valid, then the shoaling of the Panama Seaway should mark the end of the negative correlation between the oxygen isotope and carbonate records.

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Evidence for the absence of biological methylation of lead in the environment

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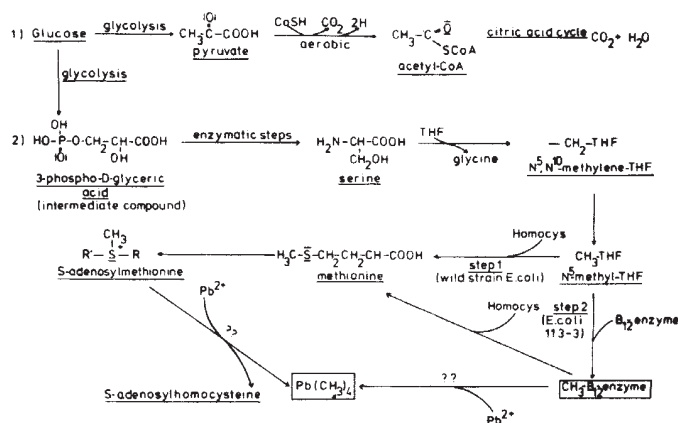
Biological methylation of arsenic and mercury by pure cultures of microorganisms such as moulds¹ and bacteria², and by 'mixed cultures of environmental systems', such as sediments³, sludges or canal mud⁴, seem to be well known processes, although there is no unequivocal evidence of the proposed pathways⁵⁻⁷. In contrast, the biomethylation of inorganic or ionic organic lead compounds has been a topic of controversy, with no conclusive explanation of the mechanism of the reactions that might lead to the corresponding alkyl lead compounds⁸⁻¹³. We report here attempts to produce leadalkyls in anaerobic and aerobic conditions. Our study was greatly helped by our use of labelled and unlabelled substrates as methyl donors and labelled and unlabelled lead compounds as acceptors. In none of numerous experiments have we found leadalkyls to be the products of biomethylation. Experiments with organic lead salts resulted in lead tetraalkyls but only as the products of chemical reactions.

We started with the assumption that if microorganisms could really convert inorganic Pb(II) compounds and organic Pb(IV) salts, such as PbMe₃OAc, PbEt₃OAc and PbEt₃Cl, into the corresponding leadalkyls, a biochemical carbanion transfer reaction should occur in the bacterial cell¹⁴⁻¹⁶. We therefore used mixed cultures and an enrichment culture of methanogenic bacteria for the anaerobic experiments, and a mixed culture of aerobic bacteria and the *Escherichia coli* auxotroph 113-3 for the aerobic experiments. The enzymatically bound CH₃-cobalamin (Fig. 1) formed from various substrates by these organisms could act as a carbonium donor in the formation of methane and methionine, respectively, or possibly as a biological 'Grignard intermediate', transferring a carbanion (CH₃⁻) to lead compounds, with leadalkyls as the final products. The criterion for the metabolic activity was the formation of CH₄/CO₂ and CO₂ by the anaerobic and aerobic bacteria, respectively. The criterion for the metabolic activity of *E. coli* 113-3 was methionine formation.

Experiments were carried out in bioreactors attached to leadalkyl collectors for trapping the alkyls blown out with the CH₄/CO₂ gas mixture produced by methanogenic bacteria, or with the air stream fed into the reactor during incubation of aerobic bacteria. The bottom of the collector was equipped with a rubber septum for sampling using syringes. Three analytical methods were applied (Fig. 2). In tracer-free experiments a gas chromatograph in combination with an atomic spectrophotometer (GC-AAS) served as the leadalkyl detector system. Samples from the collector were analysed daily by direct injection of the liquid from the collector into the gas chromatograph. Leadalkyls possibly adsorbed on the reactor wall, the bacterial mass or the sediments were also extracted after incubation and analysed by GC-AAS. Sediments and nutrient solutions were analysed for non-volatile partially biomethylated intermediates such as PbMe₃⁺ and PbMe₂²⁺ by TLC¹⁷.

The incubation experiments with ¹⁴C-labelled substrates and ²¹⁰Pb enabled us to use two further detection procedures. After refluxing the collector liquid to blow out ¹⁴CH₄ and ¹⁴CO₂ absorbed in the cooled liquid, one sample was analysed by GC-AAS and another by liquid scintillation counting. If lead had been methylated there would have been a relationship between the count rate remaining after refluxing and the peak

Presumed aerobic pathways to tetraalkyllead



Presumed anaerobic pathway to tetraalkyllead

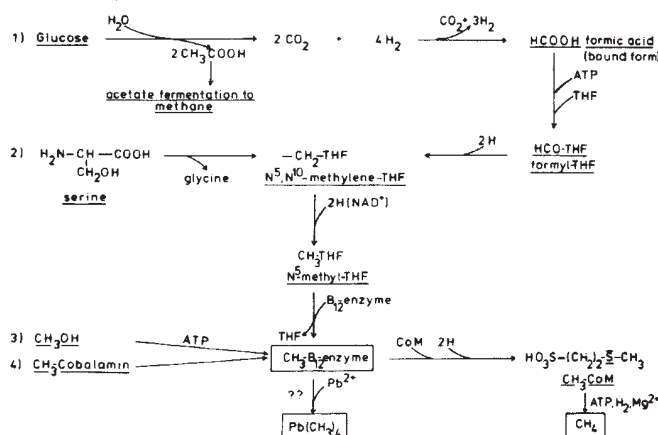


Fig. 1 The biosynthesis of the CH₃-B₁₂-enzyme from various substrates and the hypothetical methyl group transfer to lead in anaerobic and aerobic conditions.

height found by GC-AAS. In the ²¹⁰Pb-tracer experiments with unlabelled substrates, a multi-channel analyser was used as leadalkyl detector, and in the ²¹⁰Pb-tracer experiments with the labelled substrates we used the multi-channel analyser and liquid scintillation counting as detector systems. Here, too, a relationship between the ²¹⁰Pb and ¹⁴C radioactivity would have to be found if lead compounds were methylated.

In none of our experiments were leadalkyls or partially methylated intermediates detected as the products of biomethylation. Analysis of the collector liquid in the incubation experiments with inorganic lead and the ¹⁴C-labelled substrates after refluxing revealed no Pb¹⁴Me₄ peak, but there was a residual count rate, possibly representing a trace of Pb¹⁴Me too low to be detectable by the GC-AAS. However, experiments with ²¹⁰Pb²⁺ as the methyl group acceptor and ¹⁴C-serine and ¹⁴C-methanol, as the methyl group donors, respectively, proved conclusively that this residual count rate was not due to Pb¹⁴Me. As before, there was a residual ¹⁴C-count rate, but no corresponding ²¹⁰Pb radioactivity was detectable. The incubation experiments with ²¹⁰Pb²⁺ and the unlabelled substrates have verified these results. At no time did the analysis of the leadalkyl trap with the multi-channel analyser exceed 135 counts per h. This count rate represented the 10⁻⁸ part of the count rate produced by 100 μCi ²¹⁰Pb radioactivity added to the culture initially.

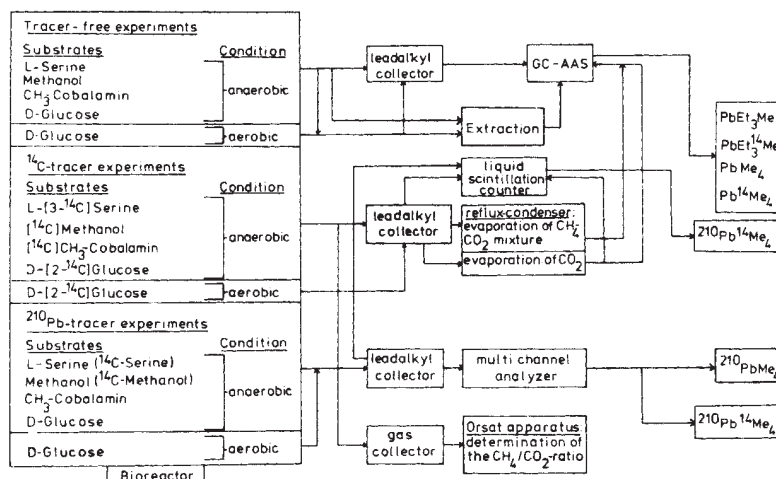
However, incubation with PbMe₃OAc, PbEt₃OAc and the substrates summarized in Table 1 yielded PbMe₄ and PbEt₄ (Fig. 3a). However, as no PbMe₃¹⁴Me or PbEt₃¹⁴Me was found,

Table 1 Incubation mixtures for the anaerobic and aerobic experiments at 37 °C

		Substrates								Nutrient medium
		L-Serine ¹⁴ C		Methanol ¹⁴ C		CH ₃ -cobalamin ¹⁴ C		D-Glucose ¹⁴ C		
		g l ⁻¹	μCi l ⁻¹	ml l ⁻¹	μCi l ⁻¹	g l ⁻¹	μCi l ⁻¹	g l ⁻¹	μCi l ⁻¹	
Anaerobic experiments										
River sediment +	Pb ²⁺ -210	5	—	10	—	0,5	—	5	—	A
	Pb ²⁺	5	20	10	20	0,5	—	5	—	
	PbMe ₃ OAc	5	—	10	—	0,5	—	5	—	
	PbEt ₃ OAc	5	100	10	20	0,5	50	5	5	
	PbEt ₃ Cl	5	20	10	20	0,5	50	5	5	
Sewage sludge +	Pb ²⁺ -210	5	20	10	—	0,5	—	5	—	A
	Pb ²⁺	5	20	10	20	0,5	—	5	—	
	PbMe ₃ OAc	5	20	10	—	0,5	—	5	—	
	PbEt ₃ OAc	5	20	10	20	0,5	50	5	5	
	PbEt ₃ Cl	5	20	10	20	0,5	50	5	5	
<i>Methanosarcina barkeri</i> +	Pb ²⁺ -210	—	—	10	20	—	—	—	—	A
	PbEt ₃ OAc	—	—	10	20	—	—	—	—	
	PbEt ₃ Cl	—	—	10	20	—	—	—	—	
Marine sediment + Pb ²⁺ -210		—	—	10	—	—	—	—	—	
Aerobic experiments										
River sediment +	Pb ²⁺ -210	—	—	—	—	—	—	5	—	B
	Pb ²⁺	5	—	—	—	—	—	5	5	
	PbMe ₃ OAc	5	—	—	—	—	—	5	5	
	PbEt ₃ OAc	5	—	—	—	—	—	5	5	
<i>E. coli</i> 113-3 +	Pb ²⁺	0,3	100	—	—	—	—	5	—	C
	PbMe ₃ OAc	1,0	20	—	—	—	—	5	—	
	PbEt ₃ OAc	1,0	20	—	—	—	—	5	—	

Incubation mixtures were 200g sediment or sludge, 50 ml enrichment culture or 5 ml pure *E. coli* culture, 60 mg unlabelled Pb compounds or 100 μCi ²¹⁰Pb(NO₃)₂ (specific activity 1 mCi per 0.016 mg Pb), the substrates and 800 ml nutrient medium. Nutrient medium A (per l): K₂HPO₄ (1 M), 3.7 ml; NH₄Cl (10%), 4 ml; MgCl₂·6 H₂O (16.6%), 1 ml; FeCl₂·4 H₂O (0.72%), 2 ml; Wolfe's minerals, 20 ml; Wolfe's vitamins, 20 ml; NaOOCCH₃·3 H₂O, 1.7 g; NaOOCCH₃, 2 g; NaHCO₃, 2 g; cysteine-HCl·H₂O, 0.5 g. pH of final mixture 7.0. Nutrient medium B (per l): K₂HPO₄, 7 g; KH₂PO₄, 3 g; Na-citrate·(5.5 H₂O), 0.55 g; (NH₄)₂SO₄, 2 g; D,L-aspartic acid, 1 g; D-glucose, 5 g; MgSO₄, 0.05 g; NaCl, 1 g. pH of final mixture 7.4. Nutrient medium C (per l): as medium B, but with vitamin B₁₂, 1 μg; D,L-homocysteine, 0.3 g.

Fig. 2 Analytical methods for the determination of leadalkyls possibly formed by anaerobic or aerobic incubation in the presence of various substrates and lead compounds. During the incubation experiments leadalkyl collector used 4 ml ethanol as absorbent liquid, -30 °C; extraction of the incubation mixture was with 20 ml ligroin (b.p. 60–80 °C); GC-AAS 10 μl; detection limit 0.09 μg Pb per ml extract. Liquid scintillation counting used 0.5 ml extract and 15 ml scintillation gel; background count rate was 13 c.p.m.



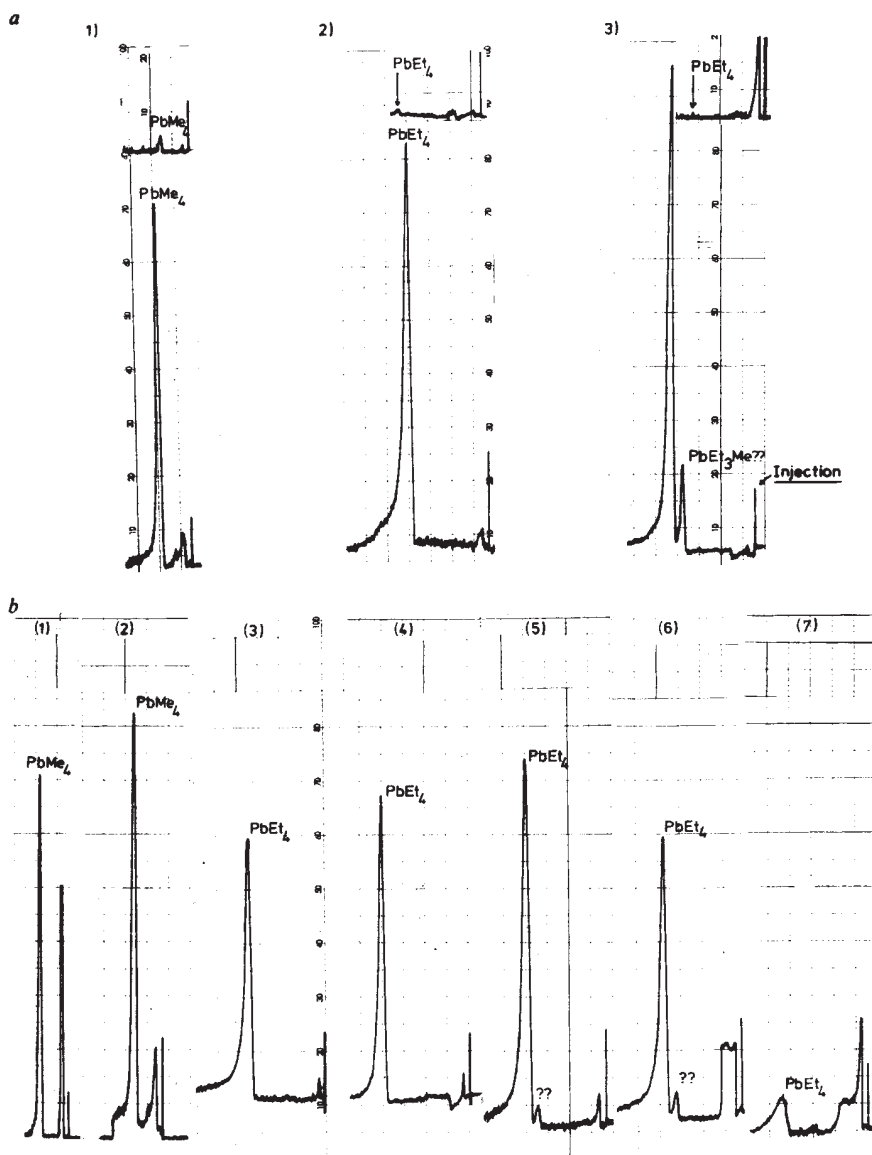


Fig. 3 The conversion of $PbMe_3OAc$, $PbEt_3OAc$ and $PbEt_3Cl$ in *in vivo* and *in vitro* conditions. *a*, Results of *in vivo* incubation of organic Pb(IV) salts with sewage sludge and L-serine substrates for 24 h. (1) Sludge + $PbMe_3OAc$; (2) sludge + $PbEt_3OAc$; (3) sludge + $PbEt_3Cl$. For comparison, blanks due to chemical side reactions in distilled water are given at the top. *b*, Results after the *in vitro* reaction (37 °C) between organic Pb(IV) salts and sulphur-containing compounds such as Na_2S , cysteine and methionine for 24 h (7: 96 h). Reaction mixture was 200 ml tridistilled water (de-aerated by boiling and gasing with N_2), 10 mg Pb compound, 10 mg sulphur compound. (1) $PbMe_3OAc$ + sulphide; (2) $PbMe_3OAc$ + cysteine; (3) $PbEt_3OAc$ + sulphide; (4) $PbEt_3OAc$ + cysteine; (5) $PbEt_3Cl$ + sulphide; (6) $PbEt_3Cl$ + cysteine; (7) $PbEt_3Cl$ + methionine.

the incubation products could not be the result of biological methyl transfer from the substrates to the corresponding ionic organic lead compounds. Moreover, tests with sulphur-containing substances have unambiguously verified the results of the important finding of Jarvie *et al.*¹² that $PbMe_4$ and naturally $PbEt_4$ are the result only of a chemical conversion induced by sulphide. Nevertheless, not only is 'free sulphide' involved in the reaction but also amino acids containing 'bound sulphide', such as cysteine and glutathione (Fig. 3b). On the other hand, sulphur-free amino acids, amino acids with blocked S-functions (methionine; Fig. 3b, chromatogram 7) or compounds with S-functions, such as thiourea, had no effect on the chemical conversion of the organic lead salts.

Incubation with $PbEt_3Cl$ (Fig. 3a) and $PbEt_4$ seemed to produce $PbEt_3Me$ which could be considered a product of biomethylation. However, the appearance of the presumed $PbEt_3Me$ compound even in the absence of any methylating agent, with only sulphide present (Fig. 3b, chromatograms 5 and 6), proved that the peak presumed $PbEt_3Me$ in Fig. 3a was not the result of biological methyl transfer but corresponds to an impurity. Tracer experiments have unambiguously supported these findings.

We therefore conclude that there is no basis for the assumption that biological methylation of Pb(II) occurs in the

environment, whereas sulphide-induced chemical conversion of organic Pb(IV) salts into leadalkyls is possible.

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Glycosaminoglycan synthesis by the apical ectodermal ridge of chick limb bud

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The apical ectodermal ridge (AER) along the distal periphery of the embryonic chick limb bud seems to have a decisive influence on the development of subjacent mesenchymal cells, preventing cartilage differentiation but ensuring the outgrowth and formation of distal limb structures¹⁻⁵. It has been suggested that when mesenchymal cells leave the AER's influence, they undergo an intimate cell-cell interaction that triggers chondrogenic differentiation by elevating cyclic AMP levels⁶⁻⁸. Moreover, the AER may inhibit the differentiation of subjacent mesenchymal cells by causing them to secrete a considerable quantity of hyaluronate which by accumulating extracellularly may prevent the cell-cell interaction necessary to trigger differentiation⁹. Here, we have examined the synthesis of hyaluronate and other glycosaminoglycans by the AER and by limb and non-limb ectodermal tissues that do not promote limb bud outgrowth^{1,5,10,11}. We find that there is a selective and substantial increase in the amount of hyaluronate produced by the AER compared with the non-inductive ectodermal tissues.

Distal wing bud tips composed of the sub-ridge mesoderm capped by the AER and surrounded dorsally and ventrally by ectoderm were cut away from stage 25 embryos¹² of White Leghorn chicks (see Fig. 1a and ref. 4), and the ectodermal jackets (Fig. 1b) were separated intact from the sub-ridge mesoderm (Fig. 1c) following brief treatment with trypsin⁴. The AER was then surgically separated from the dorsal/ventral limb ectoderm by cutting with fine knives along the dotted line

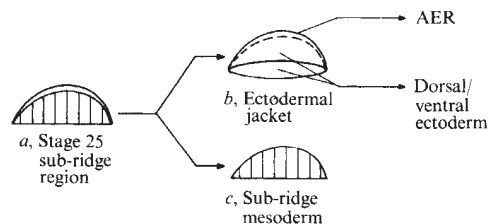


Fig. 1 Diagram of the method used to obtain AERs and dorsal/ventral limb ectoderm from the sub-ridge region of a stage 25 limb bud. Ectodermal jackets (b) were separated intact from the sub-ridge mesoderm (c) following a brief treatment with trypsin⁴, and the AER was then surgically separated from the dorsal/ventral limb ectoderm by cutting with fine knives along the dotted line indicated in b.

indicated in Fig. 1b. These procedures cleanly separate the ectodermal tissues from the sub-ridge mesoderm, and no adherence of mesodermal tissue to the isolated AERs and dorsal/ventral ectoderm is detectable with the dissecting microscope. In addition, non-limb ectodermal tissue surrounding the flank region of stage 25 embryos was removed with the aid of trypsin. The isolated AERs, the pieces of dorsal/ventral limb ectoderm and the non-limb flank ectoderm were incubated separately at 37 °C for 90 min, 17 h or 24 h in F12X medium containing 10% fetal calf serum and 100 μ Ci ml⁻¹ of ³H-glucosamine. For the 90-min incubation 150–200 AERs were used, and 30–50 AERs for the longer incubations. After incubation, glycosaminoglycans (GAGs) were extracted from the tissues and medium and the types of GAGs synthesized were determined as described in Table 1 legend.

Table 1 shows the relative amounts of the various ³H-glucosamine-labelled GAGs accumulated by the AER, dorsal/ventral limb ectoderm and flank ectoderm after the three labelling periods. The only major difference in the pattern of GAG accumulation between the AER and dorsal/ventral limb ectoderm after each labelling period is in the relative amount of hyaluronate; the hyaluronate accumulated by the AER (~8%) is about twice that accumulated by the dorsal/ventral ectoderm (~4%). In contrast, there is little, if any, difference in the

Table 1 Relative amounts of the various ³H-glucosamine-labelled components accumulated by the AER, dorsal/ventral limb ectoderm and flank ectoderm after various labelling periods

Labelling period	Tissue	Hyaluronate		Chondroitin sulphate		Other GAGs		Glycoprotein	
		%	Relative to AER*	%	Relative to AER*	%	Relative to AER*	%	Relative to AER*
90 min	AER	9.4 ± 0.7	—	27.7 ± 3.0	—	15.1 ± 2.8	—	47.8 ± 4.9	—
	Dorsal/ventral limb ectoderm	3.6 ± 0.4	0.38	28.3 ± 2.5	1.02	18.0 ± 2.1	1.19	50.2 ± 4.9	1.06
	Flank ectoderm	8.1 ± 1.7	0.86	27.6 ± 4.8	1.00	15.1 ± 3.8	1.00	49.3 ± 9.6	1.04
17 h	AER	7.3 ± 1.1	—	15.0 ± 1.8	—	14.3 ± 3.7	—	63.6 ± 0.6	—
	Dorsal/ventral limb ectoderm	3.8 ± 0.7	0.52	15.2 ± 1.3	1.01	14.4 ± 1.3	1.01	66.7 ± 0.8	1.05
	Flank ectoderm	4.7 ± 2.4	0.64	10.9 ± 2.5	0.73	17.9 ± 2.2	1.25	66.7 ± 2.5	1.05
24 h	AER	8.1 ± 0.9	—	15.9 ± 1.7	—	17.8 ± 0.4	—	58.2 ± 2.9	—
	Dorsal/ventral limb ectoderm	3.9 ± 0.8	0.48	15.5 ± 2.1	0.97	16.7 ± 0.6	0.94	63.9 ± 2.3	1.10
	Flank ectoderm	6.6 ± 1.8	0.81	10.6 ± 1.6	0.67	19.8 ± 0.4	1.11	63.2 ± 3.9	1.09

After the appropriate labelling period, GAGs were extracted from the tissue and medium as previously described¹⁵. Briefly, the tissue and medium were sonicated, and an aliquot of the sonicate removed for DNA determination¹⁶. The sonicates were boiled, digested with Pronase (Calbiochem), treated with ice-cold trichloroacetic acid, and the acid-soluble material was extensively dialysed to remove unincorporated isotope. The dialysates were lyophilized or evaporated to dryness, and dissolved in distilled water. The effectiveness of the isolation procedure in removing unincorporated isotope was monitored in each experiment by subjecting ³H-glucosamine-containing medium which was incubated in the absence of tissue to the extraction procedure. The amount of ³H-glucosamine-labelled hyaluronate was determined as previously described¹⁷ using leech hyaluronidase (hyaluronic acid-B1, 3 hydrolase; Biometrics), an enzyme which specifically degrades hyaluronate^{18,19}. Briefly, after treatment of an aliquot of the ³H-glucosamine-labelled samples with the enzyme, the amount of radioactivity associated with leech hyaluronidase-sensitive and -resistant material was determined after precipitation of undegraded GAGs with cetylpyridinium chloride (CPC)¹⁷. Data were corrected for no enzyme control values, that is, the amount of untreated ³H-glucosamine-labelled material soluble in CPC. The amount of untreated ³H-glucosamine-labelled material soluble in CPC was taken to represent labelled polysaccharide chains of glycoproteins (see ref. 17). The amount of ³H-glucosamine-labelled chondroitin sulphate was determined as previously described¹⁷ using chondroitinase ABC (Miles), an enzyme which degrades the isomeric chondroitin sulphates as well as hyaluronate²⁰. Aliquots of the GAG samples were treated with the enzyme, and the amount of radioactivity associated with chondroitinase-sensitive and -resistant material determined after precipitation with CPC¹⁷. As chondroitinase ABC degrades hyaluronate as well as the isomeric chondroitin sulphates, the amount of hyaluronate (leech hyaluronidase-sensitive GAGs) was subtracted from the amount of chondroitinase-sensitive GAGs to determine the amount of radioactivity associated with chondroitin sulphates¹⁷. The other GAGs referred to represent ³H-glucosamine-labelled GAGs resistant to degradation with chondroitinase ABC. About 50% of the chondroitinase ABC-resistant material produced by both the AER and dorsal/ventral limb ectoderm has been found to be sensitive to degradation by nitrous acid, and thus probably represents heparan sulphate. Values (%) represent the mean of three (90 min and 24 h) or two (17 h) determinations ± s.e.m.

* The relative amount of the appropriate ³H-glucosamine-labelled component accumulated by the dorsal/ventral limb ectoderm or flank ectoderm was divided by the relative amount of that component accumulated by the AER.

Table 2 Total amounts of the various ^3H -glucosamine-labelled components accumulated by the AER, dorsal/ventral limb ectoderm and flank ectoderm after various labelling periods

Labelling period	Tissue	Hyaluronate (c.p.m. per μg DNA)	Chondroitin sulphate (c.p.m. per μg DNA)	Other GAGs (c.p.m. per μg DNA)	Glycoprotein (c.p.m. per μg DNA)
90 min	AER	703	1,966	1,046	3,810
	Dorsal/ventral limb ectoderm	234	1,859	1,168	3,613
	Flank ectoderm	196	654	347	1,467
17 h	AER	8,852	18,409	18,441	79,385
	Dorsal/ventral limb ectoderm	2,241	8,411	8,332	37,616
	Flank ectoderm	1,677	3,645	5,434	20,778
24 h	AER	12,209	23,574	26,688	86,962
	Dorsal/ventral limb ectoderm	2,821	12,033	13,420	50,797
	Flank ectoderm	2,958	4,872	9,267	29,933

Values are the means of three (90 min and 24 h) or two (17 h) separate experiments.

Table 3 Amounts of the various ^3H -glucosamine-labelled components accumulated by the dorsal/ventral limb ectoderm and flank ectoderm relative to the amounts accumulated by the AER

Labelling period	Tissue	Hyaluronate	Chondroitin sulphate	Other GAGs	Glycoprotein
90 min	Dorsal/ventral limb ectoderm	0.37 ± 0.08	0.98 ± 0.06	1.17 ± 0.17	1.01 ± 0.11
	Flank ectoderm	0.29 ± 0.05	0.33 ± 0.004	0.34 ± 0.03	0.35 ± 0.06
17 h	Dorsal/ventral limb ectoderm	0.25 ± 0.12	0.45 ± 0.10	0.45 ± 0	0.47 ± 0.08
	Flank ectoderm	0.18 ± 0.17	0.19 ± 0.13	0.30 ± 0.01	0.25 ± 0.08
24 h	Dorsal/ventral limb ectoderm	0.29 ± 0.05	0.54 ± 0.04	0.52 ± 0.05	0.62 ± 0.06
	Flank ectoderm	0.33 ± 0.16	0.26 ± 0.09	0.41 ± 0.15	0.37 ± 0.13

The amount of the appropriate ^3H -glucosamine-labelled component (c.p.m. per μg DNA) accumulated by the dorsal/ventral limb ectoderm or flank ectoderm was divided by the amount (c.p.m. per μg DNA) of that component accumulated by the AER. Values are the means of three (90 min and 24 h) or two (17 h) separate experiments $\pm$ s.e.m.

relative amounts of the other ^3H -glucosamine-labelled components accumulated by the two tissues. The difference in the relative amount of hyaluronate accumulated by the AER compared with the flank ectoderm is not as great as that between the AER and dorsal/ventral limb ectoderm. However, as described below, the total amount of hyaluronate accumulated by the flank ectoderm during all labelling periods is considerably less than the total amount accumulated by the AER. Also, the pattern of GAG accumulation by the isolated AERs is strikingly different from the pattern of accumulation by the mesenchymal cells directly subjacent to the AER (see ref. 9).

Table 2 shows the total amounts (c.p.m. per μg DNA) of the various ^3H -glucosamine-labelled GAGs accumulated by the different ectodermal tissues, and Table 3 summarizes the differences in the total amounts of the various ^3H -glucosamine-labelled components accumulated by the dorsal/ventral limb ectoderm and flank ectoderm and the AER. During a 90-min labelling period, the AER accumulates almost threefold more hyaluronate than the dorsal/ventral limb ectoderm, whereas there is little, if any, difference in the total amounts of other ^3H -glucosamine-labelled components accumulated by the two tissues. Thus, after a 90-min labelling period there is a selective quantitative increase in the amount of hyaluronate produced by the AER over the immediately adjacent dorsal/ventral limb ectoderm. After either a 17- or 24-h labelling period, there is an increase in the accumulation of all ^3H -glucosamine-labelled materials by the AER compared with the dorsal/ventral limb ectoderm. Note, however, that whereas the amount of chondroitin sulphate, chondroitinase-resistant GAGs and glycoprotein produced by the AER was doubled compared with the dorsal/ventral limb ectoderm, the amount of hyaluronate accumulated increased fourfold. Thus, there is a striking disproportionate increase in hyaluronate accumulation by the AER compared with the dorsal/ventral limb ectoderm. Finally, after either short (90 min) or long labelling (17 and 24 h) periods, there is a considerable reduction (three- to fivefold) in the accumulation of all ^3H -glucosamine-labelled components by the flank ectoderm compared with the AER (Tables 2, 3).

Obviously, our results do not prove that the substantially

larger amount of hyaluronate produced by the AER than by the other ectodermal tissues is involved in the unique outgrowth-promoting effect of the AER. The possibility that the hyaluronate produced by the AER is involved in the negative differentiative effect the AER exerts on subjacent mesenchymal cells is, however, particularly intriguing. We have previously suggested that the AER may inhibit cartilage differentiation by causing subjacent mesenchymal cells to secrete large amounts of hyaluronate which prevent a cell-cell interaction necessary to trigger differentiation and/or which feed back on the cells, inhibiting their production of cartilage matrix components⁹. It is conceivable, therefore, that the hyaluronate produced by the AER may be involved in inhibiting differentiation by acting on the immediately subjacent mesenchymal cells, causing them to maintain their own high rate of hyaluronate synthesis, or perhaps by simply contributing to the hyaluronate-rich matrix surrounding the subjacent mesenchymal cells. Notably studies by Toole^{13,14} indicate that exogenous hyaluronate exerts an inhibitory effect on *in vitro* chondrogenic differentiation.

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Induction of anti-H-2 antibodies without alloantigen exposure by *in vivo* administration of anti-idiotypic

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Anti-idiotypic reagents have been used recently to manipulate both idiotype (ID) expression and antigen-binding antibody activity of immunized animals¹⁻¹¹. The *in vivo* administration of anti-idiotypic antibodies followed by antigen exposure has usually resulted in suppression of ID-positive antigen binding molecules⁶⁻⁸, although, in other circumstances, increased idiotype expression and antigen-binding activity has also been observed¹⁻⁵. Extension of these findings to the immune response to transplantation antigens is of obvious practical and theoretical importance. There have been several reports describing the production of anti-idiotypic reagents against alloantisera⁹⁻¹¹, although the generation of anti-ID antibodies in this way has generally been difficult to reproduce¹¹. Therefore, in the study reported here we used xenogeneic anti-idiotypic antisera raised against monoclonal anti-H-2K^k antibodies which offer the advantage of reproducibility of anti-idiotypic production¹². These anti-idiotypic reagents were produced by immunizing swine and rabbits with either purified 3-83P¹³ or 11-4.1 (ref. 14), both of which are IgG2a, k anti-H-2K^k antibodies. We found that none of these reagents detected significant levels of idiotype in anti-H-2 antisera produced against H-2K^k antigens by conventional means. However, BALB/c mice treated with these anti-idiotypes consistently produced large amounts of idiotype, and in some animals this induced response included idiotype-positive antibodies which bound to H-2K^k antigens. Thus, treatment with anti-idiotypic has been found to induce anti-H-2K^k antibodies in the absence of exposure to the H-2K^k antigen.

Immune antisera were absorbed with LPC-1 myeloma (IgG2a, k) coupled to Sepharose to remove all anti-normal mouse immunoglobulin. Purified anti-3-83P or anti-11-4.1 antibodies were obtained by specific adsorption to and elution

Table 1 Idiotype expression on serum antibodies from BALB/c mice immunized with anti-idiotypic antibodies or alloantigen

Group	Treatment	HAI titre (log ₂) of sheep red blood cells coated with:			
		3-83P ID		11-4.1 ID	
		Pig α	Rabbit α	Pig α	Rabbit α
(1)	BALB.K skin graft and spleen cells	≤ 1	≤ 1	≤ 1	≤ 1
(2)	Rabbit anti-3-83P antibodies	8-9	6-8	1-2	≤ 1
(3)	Pig anti-11-4.1 antibodies	1-2	≤ 1	5-6	5-6
(4)	Rabbit anti-normal mouse immunoglobulin	≤ 1	≤ 1	≤ 1	≤ 1
(5)	Pig anti-normal mouse immunoglobulin	1-2	<1	1-2	<1

In group 1, BALB/c mice were immunized against H-2^k antigens by a BALB.K tail skin graft and subsequent boosting i.p. with 2×10^7 BALB.K spleen cells per mouse. Other groups of BALB/c mice were treated on day 0 and 3 with 20 μ g of the antibodies injected i.p. in saline. On day 42, mice were bled and separate pools of sera from each group of mice were assayed for both idiotype expression and for anti-H-2K^k activity (Fig. 1). Idiotype levels were determined using a haemagglutination inhibition assay (HAI) as described elsewhere¹.

from Sepharose columns bearing the appropriate idiotype. We have previously demonstrated that these purified anti-idiotypic reagents detect unique idiotype determinants on the monoclonal antibodies but do not cross-react with each other or with a series of other monoclonal anti-H-2K^k antibodies, some of which share identical specificities¹². The effects of *in vivo* administration of these anti-ID reagents on both the expression of ID and the generation of H-2K^k antigen binding molecules were examined.

In the first experiment (Table 1) groups of BALB/c mice were treated with alloantigen (C3H skin graft and lymphoid cells), anti-idiotypic antibodies, or anti-normal mouse immunoglobulin (eluted from LPC-1 coupled to Sepharose). The animals were bled on day 42 and a serum pool from each group tested for idiotype expression in a haemagglutination inhibition assay (HAI; ref. 15). The pool of anti-H-2K^k alloantiserum (cytotoxic titre 1:400) did not express detectable levels of ID, which confirmed previous results on pools of conventional anti-H-2^k antisera made in BALB/c and other strains of mice¹². In contrast, treatment with purified anti-idiotypic antibodies (20 μ g intraperitoneally (i.p.), days 0 and 3) resulted in the

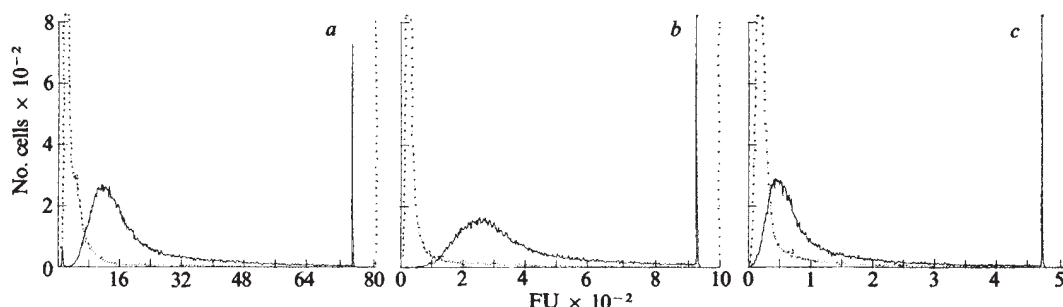


Fig. 1 Antiserum from alloimmunized and anti-idiotypic treated mice were examined for anti-H-2K^k activity by flow cytometry. Lymph node cells from C3H (H-2K^k) or C3H.OH (H-2K^d) mice were stained with test serum from individual mice of groups 1-4 (Table 1), counterstained with a mixture of fluoresceinated goat F(ab')₂ anti-mouse IgG1 (1:32) and fluoresceinated goat F(ab')₂ anti-mouse IgG2 (1:32) and analysed in a FACS II (Becton Dickinson). Fluorescence data were collected on 5×10^4 viable cells and displayed as a 1,000-channel cell frequency histogram in which fluorescence intensity was plotted (abscissa) against cell number (ordinate). Fluorescence intensity was converted to fluorescence units (FU; ref. 17) for comparison. In a, sera from a pool of mice immunized with a C3H tail skin graft and lymphoid cells were examined for anti-H-2^k activity on lymph node cells from C3H (solid line) and C3H.OH (dotted line) mice. b, Fluorescence profile of the binding of anti-H-2K^k antibodies in an Erhlich's induced ascites from mouse 1529, immunized on day 0 and 3 with 20 μ g of purified rabbit anti-3-83P antibodies and boosted with 20 μ g of anti-idiotypic 1 week before being given the ascites tumours (group 2, Table 1). c, Profile of anti-H-2K^k binding of an ascites from mouse 1505 similarly treated with pig anti-11-4.1 anti-idiotypic antibodies (Group 3, Table 1).

induction of idiotype-positive (ID^+) molecules in the serum. The observed inhibition of haemagglutination by immune sera from anti-idiotypic treated mice was not due to an anti-pig or anti-rabbit immunoglobulin response because: (1) idiotype-positive molecules induced in mice treated with pig anti-11-4.1 antibodies were detected by both rabbit and pig anti-idiotypic agglutinators (Table 1), and (2) BALB/c mice treated with either pig or rabbit anti-normal mouse immunoglobulin produced no significant levels of idiotype-positive molecules. ID^+ molecules were detected as early as 1 week after anti-idiotypic administration, reached a plateau at ~ 3 weeks, and remained detectable for several months after immunization. Only the homologous ID was expressed after *in vivo* treatment with anti-idiotypic. For example, in group 2, the injection of anti-3-83P anti-idiotypic antibodies resulted in the production of 3-83P ID^+ but not 11-4.1 ID^+ molecules.

The ascites from anti-idiotypic treated mice were next examined for anti-H-2K^k binding activity by flow cytometry (Fig. 1). Ascites from individual immunized animals were incubated with C3H (H-2K^k) or C3H.OH (H-2K^d) lymph node cells. Fluoresceinated F(ab')₂ anti-IgG1 and

F(ab')₂ anti-IgG2 antibodies were then added to the antibody-coated cells and the stained cells analysed using the fluorescence activated cell sorter (FACS II). Figure 1 illustrates the anti-H-2K^k activity of two mice, one from group 2 (1529) and one from group 3 (1505). Anti-H-2K^k binding activity was detected in three of six mice treated with rabbit anti-3-83P anti-idiotypic and in two of five mice treated with pig anti-11-4.1. None of 10 control mice treated with either rabbit or pig anti-normal mouse immunoglobulin produced detectable levels of anti-H-2K^k antibodies (data not shown). The specificity of the antigen-binding molecules produced by the anti-idiotypic treated mice was confirmed using H-2 congenic recombinant strains of mice differing only at the H-2K^k region¹⁶. The anti-H-2 antibodies induced in these animals bound to B10.AKM (H-2K^k) but not B10.MBR (H-2K^b) lymph node cells.

To assess idiotype expression on these anti-idiotypic induced anti-H-2K^k antibodies, we examined the ability of purified anti-idiotypic antibodies or anti-normal mouse immunoglobulin to block antibody binding to C3H lymph node cells. As seen in Fig. 2, anti-normal mouse immunoglobulin had no effect on the binding to the appropriate cells of either conventional allo-

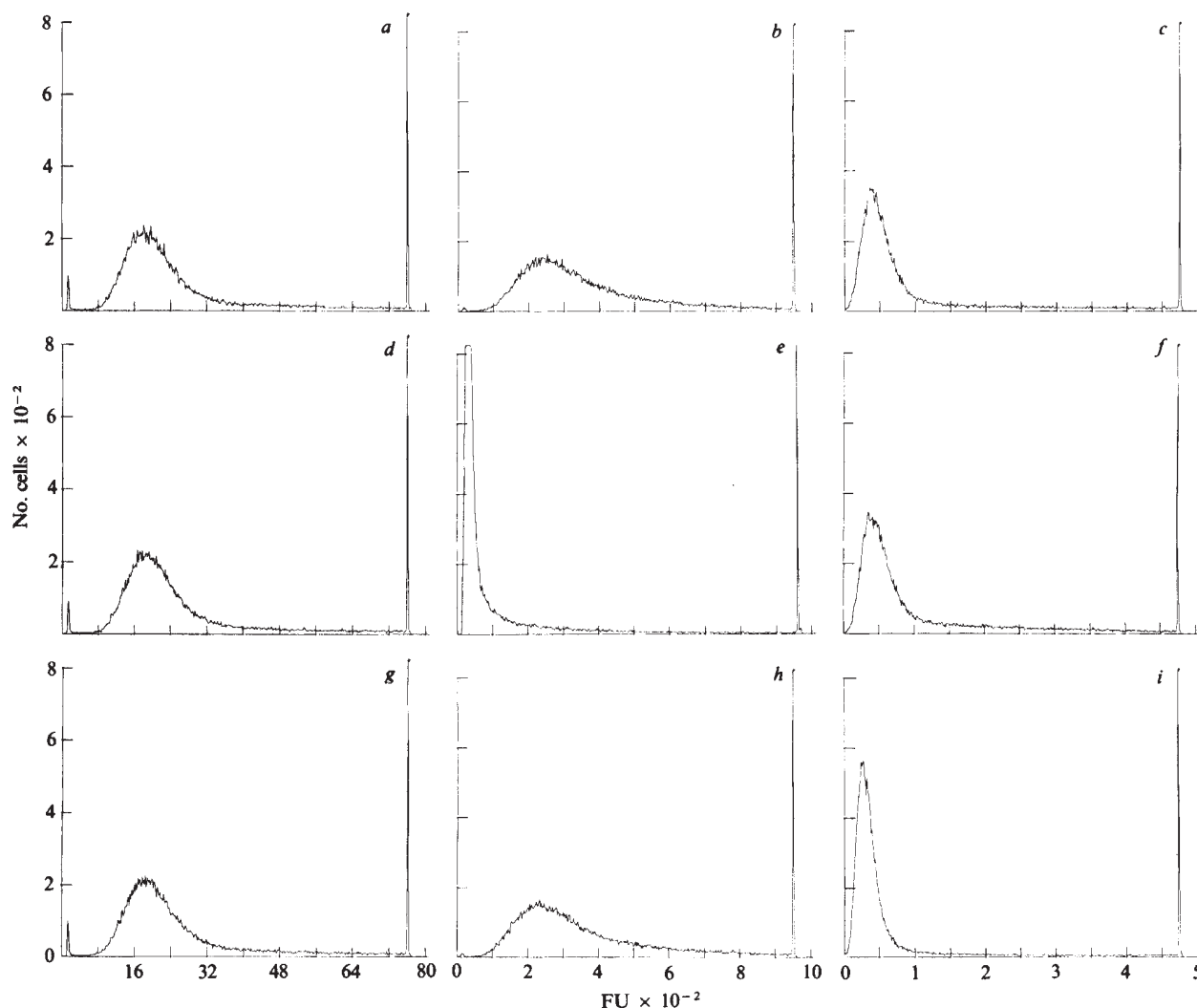


Fig. 2 Anti-H-2K^k antibodies from alloimmunized and anti-idiotypic treated mice were examined for idiotype expression by a binding inhibition assay. Serum (25 μ l) from treated mice was mixed with either 25 μ l of anti-idiotypic (100 μ g ml⁻¹) or 25 μ l of anti-normal pig immunoglobulin (100 μ g ml⁻¹). After incubation for 1 h, the test sera plus inhibitor were incubated with lymph node C3H cells and binding assessed as described in Fig. 1 legend. Binding of conventional pooled alloantisera to C3H cells was not appreciably inhibited by pig anti-normal mouse IgG (a), pig anti-3-83P (d), or pig anti-11-4.1 (g). Columns 2 (profiles b, e, and h) and 3 (profiles c, f and i) illustrate the inhibitory ability of the same pig antibodies on the binding of anti-H-2K^k antibodies induced in mice treated with rabbit anti-3-83P (1529) or pig anti-11-4.1 (1505), respectively. As the fluorescence units (FU) in each set of profiles differ, the level of inhibition must be compared with the reactivity of the serum with the congenic H-2K^d cells (Fig. 1). Such comparisons indicate that $<10\%$ of the anti-H-2K^k binding activity in mice treated with anti-idiotypic remained after inhibition with the appropriate anti-idiotypic (profiles e and i).

antibodies or anti-idiotypic induced anti-H-2K^k antibodies. In addition, antigen binding of pooled, high-titre, conventionally raised anti-H-2K^k antibodies was relatively unaffected by either anti-3-83P or anti-11-4.1 anti-idiotypic antibodies.

However, anti-idiotypic antibodies completely inhibited the binding of anti-H-2K^k antibodies induced by homologous anti-ID, as shown by a shift in intensity of the labelled cells to a level equivalent to that of controls not preincubated with allo-antibodies. Specifically, purified pig anti-3-83P antibodies completely inhibited the H-2K^k antigen binding activity of antibodies induced in mouse 1529 by rabbit anti-3-83P. In contrast, anti-11-4.1 antibodies had no effect on the binding of serum antibodies from mouse 1529 but significantly inhibited the binding of antibodies induced in mouse 1505 by pig anti-11-4.1 anti-idiotypic. These results suggest that the anti-H-2K^k antibodies induced in anti-idiotypic treated mice expressed idiotopes closely related to those of the original hybridoma anti-H-2K^k reagents. However, although both the original monoclonal anti-H-2K^k antibodies were of the IgG2a subclass, the idiotype determinants were found to be expressed on immunoglobulins of both the IgG1 and IgG2 subclasses by successive adsorption to and elution from specific goat anti-mouse IgG1 and anti-mouse IgG2 coupled to Sepharose (data not shown).

The finding that anti-idiotypic reagents produced against each monoclonal anti-H-2K^k antibody recognized determinants detected on few alloantibodies or other monoclonal anti-H-2K^k antibodies of similar specificity is most consistent with a large repertoire of anti-H-2 antibodies binding a given alldeterminant. Thus, the normal immune response to H-2 antigens such as H-2K^k may include a large number of individual clones each of which expresses private, or non-cross-reactive idiotype determinants. Such polyclonality may be the reason that production of anti-idiotypic sera against pooled alloantisera has been so difficult¹¹. Despite such polyclonality, these studies have demonstrated that among the idiotype-positive molecules induced in anti-ID treated mice are molecules which bind specifically to H-2K^k antigens. The anti-H-2K^k activity induced represents expression of V region genes which are common to BALB/c mice but which are only occasionally used in the normal response to H-2K^k. Therefore, these findings are consistent with a possible sharing of variable region structures in the anti-H-2K^k monoclonal antibodies with those of alloantibodies induced by anti-idiotypic.

The induction of antigen-binding ID⁺ molecules in the absence of exposure to antigen does not usually occur in systems involving soluble antigens after treatment with heterologous anti-ID^{1,2,4}. This difference may depend in part on the increased sensitivity of detection of antigen binding activity allowed by FACS II. However, these findings may also reflect an intrinsic difference in the response to histocompatibility antigens compared with most other antigens^{9,10}. The ability of lymphoid cells to recognize H-2 antigens is of central importance in the immune response. Anti-idiotypic antibodies to monoclonal anti-H-2 antibodies may be useful as a probe for analysing the cellular interactions involved in immune responses and as a new approach for the specific manipulation of these responses.

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Cell interactions modulate embryonal carcinoma cell differentiation into parietal or visceral endoderm

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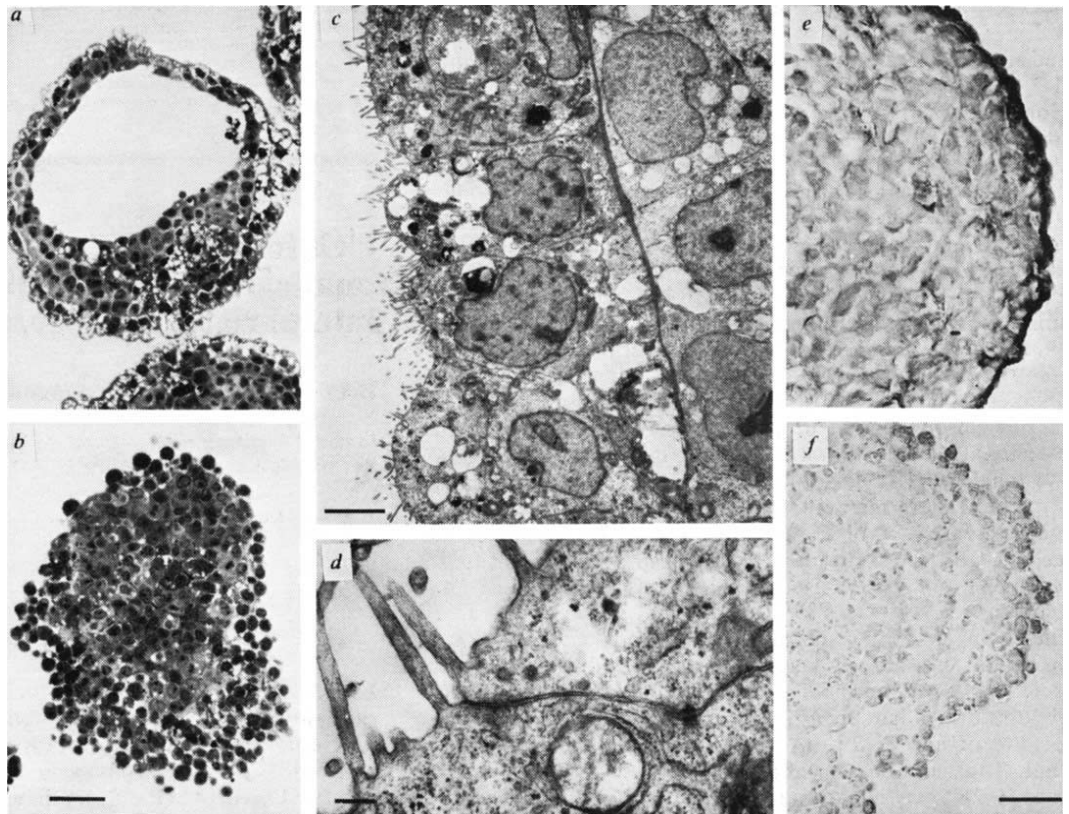
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F9 is a clonal line of mouse teratocarcinoma-derived embryonal carcinoma (EC) cells which shows very little spontaneous differentiation *in vivo* or *in vitro*¹. Recently, however, it was reported²⁻⁶ that F9 monolayers treated with retinoic acid and dibutyl cyclic AMP differentiate into an early embryonic cell type known as parietal endoderm, which is one of two distinct populations of extra-embryonic endoderm that differentiate in the normal mouse embryo shortly after implantation^{7,8}. The other population is the visceral endoderm, and the two differ not only in their morphology and location within the embryo, but also in their biochemical properties⁹⁻¹². Parietal endoderm cells, for example, do not synthesize α -fetoprotein¹³ (AFP), whereas visceral endoderm cells do¹³. There is evidence⁸ that during embryogenesis parietal and visceral endoderm are derived from a common precursor population known as the primary endoderm, and recent experiments with cultured mouse embryos¹⁴ suggest that the phenotype of these cells can be modulated by contact with different embryonic tissues. We now show that if F9 EC cultures are treated with retinoic acid when they are in the form of small aggregates, they differentiate on the outer surface cells which morphologically resemble visceral rather than parietal endoderm. In addition, the cells synthesize and secrete AFP, identified by immunoprecipitation and immunoperoxidase reactions using specific anti-AFP immunoglobulin. One interpretation of this result is that F9 cells treated with retinoic acid differentiate first into multipotent cells analogous to the primary endoderm of the normal embryo which then express either the mature parietal or visceral phenotype depending on the nature of the intercellular contact signals they receive. We therefore believe that F9 EC cells may be even more useful than previously supposed for biochemical studies on factors controlling gene expression during mammalian embryogenesis.

Small clumps of 30–50 EC cells are grown in suspension in bacteriological Petri dishes in medium containing 5×10^{-8} M retinoic acid. After 2–4 days most aggregates resemble simple embryoid bodies^{15,16} with an outer layer of endoderm surrounding a core of undifferentiated cells. These aggregates often become hollow, and for a while resemble cystic embryoid bodies¹⁵ (Fig. 1a). Later, however, this organization breaks down as an increasing proportion of the inner cells develop an endoderm-like morphology, and internal tissues analogous to embryonic ectoderm and mesoderm have never been observed, even after prolonged incubation. When aggregates cultured for 7 days are examined in the electron microscope, several kinds of endoderm cell are found. As shown in Fig. 1c and d, most

Fig. 1 Morphology of F9 cell aggregates treated with retinoic acid. *a*, Section through an aggregate of F9 cells cultured in the presence of 5×10^{-8} M retinoic acid for 7 days. Endoderm cells form an epithelial layer around the hollow core of still undifferentiated embryonal carcinoma cells. *b*, Section through an aggregate of F9 cells cultured in the presence of 1×10^{-7} M retinoic acid and 10^{-3} M dibutyryl cyclic AMP for 5 days. In these conditions the endoderm cells do not form an epithelial layer but are rounded and loosely packed, with few intercellular junctional complexes. In the electron microscope all the differentiated cells have a typical parietal endoderm morphology. Scale bar, 50 μ m for both *a* and *b*. *c*, Electron micrograph of 9f visceral endoderm-like cells present in an aggregate of F9 cells exposed to 5×10^{-8} M retinoic acid for 7 days. The cells are joined apically by junctional complexes and have numerous microvilli, endocytotic vacuoles, lysosomes and lipid droplets. These features are all characteristic of visceral endoderm of the normal mouse embryo^{7,14,17}. Scale bar, 5 μ m; *d*, Detail of a desmosome and an apical junctional complex between two adjacent visceral endoderm-like cells in an aggregate exposed to 5×10^{-8} M retinoic acid for 7 days. Such intercellular connections are not found between parietal endoderm cells in the normal embryo^{7,17}. Scale bar, 0.2 μ m. *e*, *f*, Immunoperoxidase staining for AFP on sections of F9 aggregates. *e*, Cells were cultured for 5 days in the presence of 5×10^{-8} M retinoic acid in the standard culture medium and then for 2 days in serum-free medium¹⁹. This was to avoid retention of calf serum proteins which might cross-react with rabbit anti-mouse AFP. The antibody was also pre-absorbed with Sepharose-linked calf serum proteins to reduce further the possibility of staining calf AFP which could have been endocytosed by the F9 cells. The aggregates were fixed in acidified ethanol and embedded, sectioned and reacted as described elsewhere¹³. *f*, Aggregates cultured in standard medium containing 1×10^{-7} M retinoic acid and 10^{-3} M dibutyryl cyclic AMP for 5 days were fixed, embedded, sectioned and reacted with rabbit anti-mouse AFP as described above, except that the antiserum was not pre-absorbed with calf serum proteins. No intracellular peroxidase staining is present. Scale bars, 20 μ m *e* and *f*.



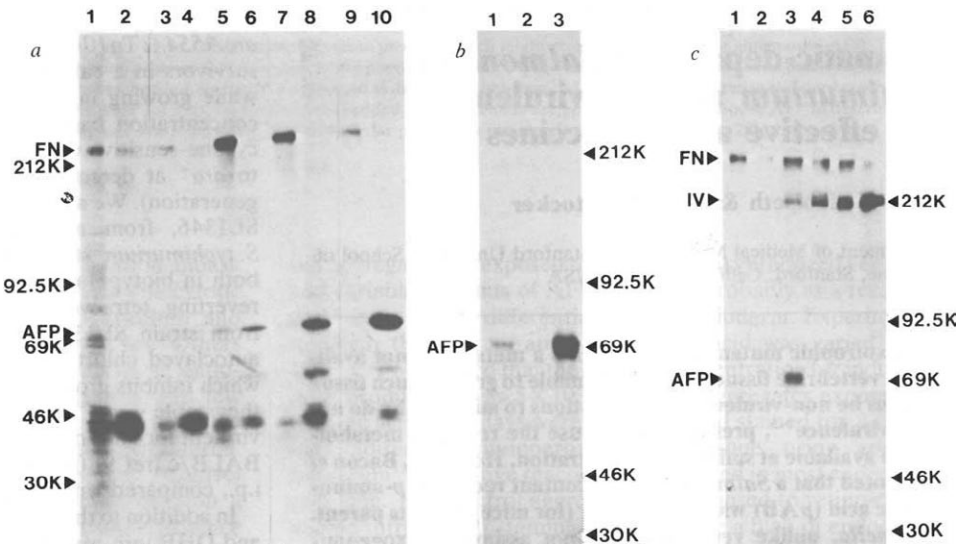
resemble visceral endoderm of the post-implantation mouse embryo^{7,12,14,17} and have numerous microvilli and pinocytotic and lysosomal vacuoles, a thin endoplasmic reticulum and prominent intercellular apical junctional complexes. Others resemble parietal endoderm^{7,12,17}, with an extensive endoplasmic reticulum engorged with secretory material and few microvilli or intercellular junctional complexes. These cells are usually associated with pools of basement membrane material. The remainder have an intermediate morphology. Control aggregates incubated without retinoic acid never develop a continuous outer layer of endoderm, although some produce small 'blebs' of visceral endoderm-like cells.

As cells with a visceral endoderm morphology are present in aggregates incubated with retinoic acid we looked for the synthesis and secretion of the visceral endoderm marker protein, AFP. Aggregates were incubated with ³⁵S-methionine, and AFP immunoprecipitated from the culture medium with specific antiserum¹³. Synthesis of AFP could be detected after only 3 days' exposure to retinoic acid, and increased up to 7 days (Fig. 2a). At this time AFP represented about 0.05% of the total newly synthesized protein secreted by F9 aggregates, compared with a value of 4% for visceral yolk sacs isolated from 11.5-day embryos, although the comparison is only approximate because of the very different cellular composition and pool sizes of yolk sacs and aggregates. Figure 2a shows that the radioactive polypeptide immunoprecipitated by anti-AFP antiserum from the medium of F9 aggregates treated with retinoic acid co-migrates on SDS-polyacrylamide gels with AFP synthesized by visceral endoderm isolated from 13.5-day mouse embryos. Control F9

cell aggregates not exposed to retinoic acid synthesized very low and variable amounts of AFP (Fig. 2a), probably as a result of some spontaneous differentiation into endoderm. Experiments in which the concentration of retinoic acid was varied over 10^{-8} – 10^{-6} M showed that the optimal concentration for inducing AFP production was 5×10^{-8} M. When aggregates exposed to retinoic acid for 7 days were sectioned and stained for AFP by the indirect immunoperoxidase technique using specific antiserum¹³, a positive reaction was obtained in most endoderm cells (Fig. 1e). Control aggregates not exposed to retinoic acid had no intracellular staining, except where a bleb of endoderm cells was present (data not shown). Because the rabbit anti-mouse AFP antiserum was pre-absorbed with calf serum proteins and did not react with calf AFP in immunodiffusion assays, we are confident that the stain is identifying endogenously synthesized AFP. However, we cannot exclude the possibility that the AFP is synthesized and secreted by only a minority of the visceral endoderm cells and is then endocytosed from the medium by the remainder.

Earlier, Strickland and Sawey⁴ failed to find evidence for AFP synthesis by F9 cells in monolayer cultures. In their experiments the cells were exposed to 1×10^{-7} M retinoic acid for 4 days. We have treated monolayers with 5×10^{-8} M retinoic acid, and observe significant AFP synthesis starting at 7 days, by which time the cultures are very dense and localized clumping between cells with endoderm and EC-like morphology has developed (Fig. 2c). In agreement with the observations of Strickland *et al.*⁶, we find that F9 monolayers exposed to both retinoic acid and dibutyryl cyclic AMP differentiate into endoderm cells

Fig. 2 Synthesis and secretion of AFP by F9 cells treated with retinoic acid. **a**, Time course of AFP synthesis by aggregates cultured in retinoic acid. Exponential cultures of F9 EC cells (from a line supplied by Colin Stewart, which had been recloned after *in vivo* passage as a tumour) were briefly trypsinized, and small aggregates resuspended in bacteriological Petri dishes in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 5×10^{-8} M retinoic acid (Sigma, added from a 10^{-4} M stock solution in alcohol and protected from light). Control aggregates were cultured without retinoic acid. The medium was changed every 48 h. After 3 days ~100 aggregates were removed every 24 h, washed twice in DMEM containing $1 \mu\text{g ml}^{-1}$ methionine and 10% dialysed FBS (labelling medium) and incubated for ~11 h in 1 ml labelling medium with $100 \mu\text{Ci } ^{35}\text{S}$ -methionine (specific activity $1,000 \text{ Ci mmol}^{-1}$; Radiochemical Centre), with and without retinoic acid. After incubation the aggregates were removed by sedimentation and 400- μl aliquots of the medium used for immunoprecipitation. Samples were diluted into 1 ml of 0.4 M NaCl, 0.005 M EDTA, 0.05 M Tris (pH 8.0) and 1% v/v NP40 (NET buffer) and 10 μl of rabbit anti-mouse AFP antiserum¹³ added. After 1.5 h at room temperature 50 μl of protein A-Sepharose (Pharmacia, resuspended in NET buffer) were added and the incubation continued with continuous mixing at 4 °C for 1.5 h. The beads were then washed by centrifugation twice in NET buffer, once in 0.01 M Tris, pH 6.8, and finally boiled for 3 min in 30 μl gel sample buffer¹⁸ (0.0625 M Tris, pH 6.8, 2% SDS, 10% glycerol, 5% β -mercaptoethanol). SDS-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli¹⁸, using 5–10% gradient slab gels. ^{14}C -labelled marker proteins (Radiochemical Centre) were included in the gel and their position is shown on the left-hand side of the figure. After electrophoresis the gels were fixed, impregnated with EN³HANCE (NEN), dried and exposed to X-ray film (Kodak SB-5) at -70 °C. Tracks 1, 3, 5, 7 and 9, proteins immunoprecipitated with anti AFP antiserum from medium of control aggregates cultured for 3, 4, 5, 6 and 7 days in the absence of retinoic acid. The medium samples contained 1.59×10^6 , 0.37×10^6 , 0.41×10^6 , 0.46×10^6 and 0.54×10^6 c.p.m. in total, acid-precipitable protein, respectively. Tracks 2, 4, 6, 8 and 10, proteins immunoprecipitated from medium of aggregates cultured for 3–7 days in the presence of 5×10^{-8} M retinoic acid. The medium contained 1.15×10^6 , 0.42×10^6 , 0.30×10^6 , 0.30×10^6 and 0.53×10^6 c.p.m. in total acid-precipitable protein, respectively. Newly synthesized AFP migrates as a radioactive polypeptide of molecular weight (MW) ~70,000. The polypeptide of MW ~57,000 probably represents an AFP degradation product (E.A., unpublished observation), while the identity of the diffuse band at MW 46,000 is unknown. This band was not observed when the antiserum was pre-absorbed with adult mouse serum (see **b** and **c**). At all times, some radioactive fibronectin MW ~265,000 is nonspecifically bound to the protein A-Sepharose. From this, it can be seen indirectly that the amount of fibronectin synthesized and/or released into the culture medium by F9 cells is reduced after exposure to retinoic acid. Similar results to those shown were obtained with a line of F9 cells obtained from D. Solter. **b**, Co-migration of AFP synthesized by F9 aggregates and by normal visceral endoderm. After 6 days in culture in 5×10^{-8} M retinoic acid, aggregates were incubated for 12 h in labelling medium containing $200 \mu\text{Ci ml}^{-1} ^{35}\text{S}$ -methionine and 5×10^{-8} M retinoic acid. Four visceral yolk sacs dissected from 13.5-day C3H/He mouse embryos were incubated for 18 h in labelling medium containing $100 \mu\text{Ci ml}^{-1} ^{35}\text{S}$ -methionine. Aliquots of the culture medium were immunoprecipitated with 4 μg of IgG prepared from rabbit anti-mouse AFP antiserum pre-absorbed with adult mouse serum proteins. Track 1, immunoprecipitate of medium from F9 aggregates; track 2, immunoprecipitate of medium from visceral yolk sacs using 10 μl of pre-immune rabbit serum; track 3, immunoprecipitate of medium from visceral yolk sacs. **c**, Synthesis of AFP by F9 monolayers incubated with and without retinoic acid and dibutyl cyclic AMP for 7 days. 1×10^4 F9 cells were seeded into 35-mm tissue culture dishes in culture medium with and without 5×10^{-8} M retinoic acid and 10^{-3} M dibutyl cyclic AMP. The medium was changed every 48 h and after 7 days replaced with 1 ml of labelling medium containing $100 \mu\text{Ci ml}^{-1} ^{35}\text{S}$ -methionine. After 13.5 h incubation the medium was removed and the cells trypsinized and counted. Cell numbers per dish were as follows: control, 5.63×10^6 ; with retinoic acid, 1.08×10^6 ; with retinoic acid and dibutyl cyclic AMP, 1.71×10^6 . The volume of culture medium used for the immunoprecipitation was adjusted to take into account the difference in cell number. Total c.p.m. in acid-precipitable protein in the medium samples were as follows: control, 6.87×10^4 (in 58 μl); with retinoic acid, 6.56×10^5 (in 300 μl); with retinoic acid and dibutyl cyclic AMP, 1.47×10^6 (in 189 μl). Medium was immunoprecipitated with either 4 μg rabbit anti-mouse AFP IgG (see **b**) or 10 μl of non-immune rabbit serum (NRS). Track 1, control medium immunoprecipitated with anti-AFP IgG; track 2, control medium immunoprecipitated with NRS; track 3, medium from cells treated with retinoic acid, immunoprecipitated with anti-AFP IgG; track 4, same medium immunoprecipitated with NRS; track 5, medium from cells treated with retinoic acid and dibutyl cyclic AMP, immunoprecipitated with anti-AFP IgG; track 6, same medium immunoprecipitated with NRS. In addition to the nonspecific precipitation of fibronectin, tracks 3–6 show the nonspecific precipitation of type IV procollagen (MW ~190,000) synthesized by F9 cells in response to retinoic acid.



which synthesized large amounts of laminin and type IV procollagen and no fibronectin (unpublished data). As shown in Fig. 2c, these cells do not synthesize AFP, a result which supports their classification as exclusively parietal endoderm. F9 cells exposed to retinoic acid and dibutyl cyclic AMP in aggregates (Fig. 1b) also develop a parietal endoderm morphology as judged by light and electron microscopy, and do not synthesize AFP or stain positively with anti-AFP antiserum using the indirect immunoperoxidase technique (Fig. 1f).

One interpretation of our results, and those of Strickland *et al.*^{4,6}, is that F9 cells exposed to retinoic acid first differentiate into endoderm cells which have the potential to express one of two alternative phenotypes. In the absence of intercellular contacts with undifferentiated cells (for example, in low density monolayer cultures) or in the presence of high levels of cyclic AMP, these endoderm cells express the parietal phenotype. However, if they remain in contact with undifferentiated EC cells (for example, in suspension aggregates, or in high-density monolayer cultures), they receive signals promoting expression of the visceral endoderm phenotype. According to this model, the first endoderm cells to differentiate from F9 EC cells are analogous to the primary endoderm cells of the 4.5-day mouse

embryo, and modulation of their phenotype in culture may therefore provide clues about the nature of the intercellular interactions thought to be involved in endoderm differentiation during normal embryogenesis¹⁴.

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Aromatic-dependent *Salmonella typhimurium* are non-virulent and effective as live vaccines

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An auxotrophic mutant which requires a metabolite not available in vertebrate tissues should be unable to grow in such tissue and thus be non-virulent. Most mutations to auxotrophy do not affect virulence¹⁻³, presumably because the required metabolites are available at sufficient concentration. However, Bacon *et al.*^{1,2} noted that a *Salmonella typhi* mutant requiring *p*-aminobenzoic acid (*p*AB) was less virulent (for mice) than its parent. *Salmonella*, unlike vertebrates, cannot assimilate exogenous folate and must synthesize it from *p*AB; the virtual absence of *p*AB from vertebrate tissues is shown by the efficacy of sulphonamide chemotherapy. Yancey *et al.*⁴ reported reduced virulence for a *S. typhimurium* mutant with a requirement for 2,3-dihydroxybenzoate (DHB), the precursor of the bacterial iron-acquisition compound, enterochelin. As DHB is not a known vertebrate metabolite, it would be expected to be absent from mouse tissues. *Salmonella* synthesize *p*AB and DHB from chorismate, the final product of the aromatic biosynthetic (*aro*) pathway. A complete block at any step of this pathway should make *S. typhimurium* auxotrophic for two compounds not available in vertebrate tissues, and thus non-virulent. We describe here the use of a tetracycline-resistance transposon, Tn10 (refs 5, 6), inserted in gene *aroA* to produce non-reverting, aromatic-requiring derivatives of virulent *S. typhimurium* strains. These derivatives were virtually non-virulent; their use as live vaccines conferred excellent protection against challenge with a virulent strain.

The *aro* transposon insertion used was *aroA554::Tn10*, in the chromosome of *S. typhimurium* strain LT2. As wild-type LT2 is only weakly pathogenic we transduced the *aroA554::Tn10* region into a virulent *S. typhimurium* strain, using phage P22 HT105/1 *int* (ref. 7) and selecting for tetracycline resistance. The virulent strain used as recipient, SL3201, is a mouse-passaged re-isolate of the genetically marked mouse-virulent *S. typhimurium* strain SL4522, of biotype FIRN⁸. Two of the transductants obtained, SL3217 and SL3218, required tryptophan, tyrosine and phenylalanine. Their additional requirements for *p*AB and DHB were observed on defined medium, made with purified agar to minimize *p*AB contamination, and containing citrate, a known iron chelator, to reduce iron availability; DHB was not needed if the medium was supplemented with ferrous sulphate. Strains SL3217 and SL3218 were then tested for pathogenicity by intraperitoneal (i.p.) injection of bacteria (grown in nutrient broth with tetracycline at 5 µg ml⁻¹) into adult mice of the *Salmonella*-susceptible⁹ inbred line C57BL. Mice given 3 × 10⁵ live bacteria of either of the two *aroA554::Tn10* strains showed no ill effects; the LD₅₀ for the *aro*⁺ parent strain, SL3201, was <20 bacteria.

Strains auxotrophic because of Tn10 insertion in a biosynthetic gene can regain their lost biosynthetic ability and lose tetracycline resistance by 'clean excision' or deletion of the transposon^{5,6}; for strains SL3217 and SL3218 the frequency of reversion to aromatic independence (and tetracycline sensitivity) was ~10⁻⁸ per bacterium per generation. Such *aro*⁺ revertants had regained virulence (LD₅₀ <20, i.p.). To obtain non-reverting *aro*⁻ strains we made use of the tendency of Tn10 to cause DNA alterations within the transposon itself, sometimes extending into adjacent chromosomal genes. Many such deletion or deletion-inversion events cause both loss of tetracycline resistance and inability of the affected gene to revert to

the wild-type form^{10,11}. Tetracycline-sensitive variants of the *aroA554::Tn10* transductant SL3218 were found amongst the survivors in a culture exposed to ampicillin at 2,500 µg ml⁻¹ while growing in the presence of tetracycline (5 µg ml⁻¹), a concentration bacteriostatic for cells lacking Tn10. A tetracycline-sensitive variant thus obtained, SL3235, did not revert to *aro*⁺ at detectable frequency (<10⁻¹¹ per bacterium per generation). We also derived an *aroA554::Tn10* transductant, SL1346, from a genetically marked subline, SL1344, of *S. typhimurium* strain S2337; this strain differs from SL3201 both in biotype and in that it is virulent for calves¹². A non-reverting, tetracycline-sensitive variant, SL3261, was isolated from strain SL1346 by plating on nutrient agar containing autoclaved chlortetracycline and fusaric acid, a combination which inhibits growth of tetracycline-resistant bacteria¹³. Both the stable *aroA*⁻ strains, SL3235 and SL3261, were non-virulent for mice of another *Salmonella*-susceptible inbred line, BALB/c (ref 9) (no deaths from inocula of ~3 × 10⁶ bacteria i.p., compared with LD₅₀ of <20 for the *aro*⁺ parent strains).

In addition to the requirement for aromatic amino acids, *p*AB and DHB, *aro* mutants will lack two other products of chorismate: ubiquinone, made via *p*-hydroxybenzoate, and menaquinone, made via *o*-succinylbenzoate. Lack of some chorismate-derived metabolite may account for two recently noted consequences of *aro* defect: failure to hypermodify a uridyl base in tRNA, reported by Björk¹⁴ for *aro* mutants in *Escherichia coli*, and failure to produce H₂S from thiosulphate, noted by us for *S. typhimurium* with non-leaky blocks at *aroA* or *aroD*. We considered the possibility that some such consequence of *aro* defect might account, in part or entirely, for the non-virulence of *aro*⁻ strains of *S. typhimurium*. However, a preliminary experiment showed that a large inoculum (2 × 10⁶ bacteria i.p.) of the non-virulent *aro*⁻ strain, SL3261, caused fatal infections in most BALB/c mice provided with drinking water containing both *p*AB and DHB; the same inoculum caused no apparent ill effects in mice which received *p*AB alone or DHB alone, or neither benzoate. This suggests that requirement for *p*AB and for DHB each by itself suffices to make strain SL3261 non-virulent for mice maintained on a normal diet, and that inability to synthesize other products of chorismate is not relevant to the non-virulence of *aro*⁻ *S. typhimurium*.

Vaccination by injection of killed *Salmonella* confers only poor protection (reduction in mortality) against challenge with a virulent strain⁹. We therefore tested the ability of our stable, *aro*⁻ strains, given i.p. as live vaccine, to protect against later challenge with a virulent *S. typhimurium* strain, given i.p. or by feeding (Table 1). The *S. typhimurium* strain used as challenge, SL1344 (the genetically marked virulent grandparent of *aro*⁻

Table 1 Challenge by i.p. or oral administration of virulent *S. typhimurium* strain SL1344 (*aro*⁺) to BALB/c mice immunized by i.p. injection of live *aro*⁻ *S. typhimurium*

<i>aro</i> ⁻ live vaccine		Challenge by <i>aro</i> ⁺ strain SL1344
Strain	Dose	Deaths/No. tested (days to death)
a Challenge by injection of 5 × 10⁵ bacteria i.p. 34 days after live vaccine		
Controls	None	5/5 (day 2, 3, 3, 4, 5)
SL3261	2 × 10 ⁴ i.p.	1/5 (day 3)*
	2 × 10 ⁵ i.p.	0/5*
	2 × 10 ⁶ i.p.	0/5*
b Challenge by feeding 3 × 10⁷ bacteria 30 days after live vaccine		
Controls	None	5/5 (day 7, 8, 9, 9, 12)
SL3261	3 × 10 ⁴ i.p.	0/5†
	3 × 10 ⁵ i.p.	0/5†
	3 × 10 ⁶ i.p.	0/5†
SL3235	3 × 10 ⁴ i.p.	0/5†
	3 × 10 ⁵ i.p.	0/5†
	3 × 10 ⁶ i.p.	0/5†

Mice used were 15–17-week-old BALB/c males.

* All surviving mice were apparently well on day 60 after challenge.

† All mice alive and apparently well on day 120 after challenge.

live vaccine strain SL3261), is virulent for mice, given i.p. or by feeding, and for calves by the oral route¹². The mice used (the susceptible BALB/c line) were given graded i.p. inocula of one or the other stable *aro*⁻ strain and were challenged 1 month later with the virulent strain, either 5×10^5 bacteria i.p. or 3×10^7 bacteria given by feeding (on bread cubes, after a 12-h fast). Mice challenged by feeding were housed one per cage to prevent cross-infection. Each of the two *aro*⁻ strains, given i.p. in doses of 3×10^4 , 3×10^5 or 3×10^6 (five mice per dose), gave complete protection against the oral challenge (no deaths and no apparent illness in 120 days of observation), whereas all five non-immunized control mice were dead by day 12. The two larger doses (2×10^5 and 2×10^6) of the *aro*⁻ strain SL3261 protected all mice (five mice per dose) against i.p. challenge and the smallest dose used, 2×10^4 live bacteria, protected four of five mice (Table 1). Thus i.p. injection of as few as 2×10^5 live *aro*⁻ bacteria into mice of a *Salmonella*-susceptible inbred line, BALB/c, gave complete protection against later i.p. injection of 5×10^5 virulent *S. typhimurium* (that is, $>25,000$ LD₅₀).

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These results are in striking contrast to the failure of killed vaccines to protect such mice; Robson and Vas⁹ found that six i.p. doses of killed *S. typhimurium* vaccine failed to protect BALB/c (or C57BL) mice against the lethal effect of i.p. injection of even as few as 10 cells of a virulent *S. typhimurium* strain. Oral administration of live bacteria of a non-virulent *galE* mutant of *S. typhi* has recently been shown to prevent typhoid fever in school children in Egypt¹⁵. Taken together, the data suggest that live vaccines may be useful for protecting man or domestic animals against *Salmonella* infections. Our results further suggest that strains with complete, non-reverting blocks in aromatic biosynthesis may be appropriate for such purposes.

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Denervated endplates have a dual population of junctional acetylcholine receptors

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The acetylcholine receptors (AChRs) at the neuromuscular junctions of mature innervated muscle are distinguished, in part, from extrajunctional receptors of embryonic muscle fibres by their metabolic stability^{1–5} and high concentration at the tops of the postjunctional folds^{6,7}. After denervation, the rate of degradation of receptors at the neuromuscular junction progressively increases^{8–12} even though their local concentration does not change^{7,8,11}. Two alternatives suggest themselves—there may be a simultaneous, progressive increase in degradation rate of all junctional receptors after denervation, or the increase may reflect the composite behaviour of two populations of junctional receptors with different metabolic stabilities: those existing before denervation ('original AChRs') and those inserted afterwards ('new AChRs'). Our study has been designed to distinguish between these possibilities. We have now found that up to 8–10 days after denervation, the original receptors have a degradation half life of about 8 days which is indistinguishable from that of innervated junctional receptors. After that time, their degradation half life increases to about 2.5 days. However, all new AChRs, introduced at junctional sites after denervation, have a half life characteristic of extrajunctional receptors (about 1 day). Our data thus support the hypothesis of a composite population of receptors at denervated neuromuscular junctions.

The turnover rate of junctional AChRs (either denervated or innervated) was assessed by the loss of radioactivity bound to the neuromuscular junction after labelling with ¹²⁵I- α -bungarotoxin, the virtually irreversible inhibitor of the muscle's nicotinic AChR^{13,14}. The validity of this procedure has been established in previous studies^{1–3,15–19}, although a slow unbinding of α -bungarotoxin has been reported in culture³. We first compared

the degradation rate of the receptors at innervated junctions with that of the receptors at denervated junctions that were present before denervation (original AChRs). Adult mice (27–37 g) were injected with ¹²⁵I- α -bungarotoxin intravenously (tail vein), 2.5 μ g per 100 g body weight (the LD₅₀ was established to be about 3.5 μ g per 100 g). At 3 h after the injection, the right sternomastoid muscle was denervated under Nembutal anaesthesia by removing 1–2 mm of nerve as close to the muscle as possible, leaving the contralateral muscle as an innervated control. (In control animals, the specific junctional counts in the left and right muscles are not significantly different at the time of injection.) At 1–2-day intervals up to 20 days later, three to nine animals were anaesthetized and killed by intracardial perfusion with 4% paraformaldehyde. The two sternomastoid muscles were removed and stained for acetylcholinesterase²⁰ to identify the endplate band. Each muscle was

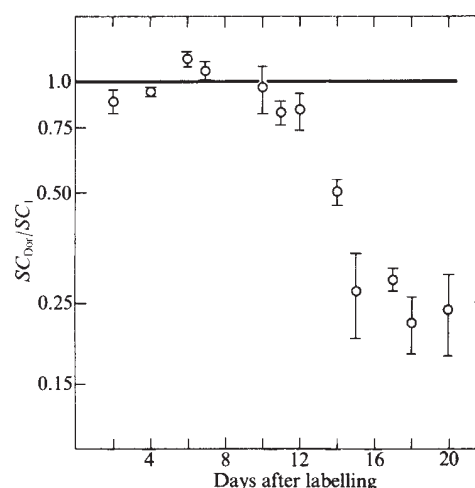


Fig. 1 Mice were injected with ¹²⁵I- α -bungarotoxin 3 h before denervation of the right sternomastoid muscle, thus labelling the original population of AChRs present before denervation. The ratio of specific endplate label of the denervated (SC_{Den}) and innervated (SC_I) muscles was determined at different days thereafter. Values become significantly different from unity (solid line) after ~10 days of denervation. Error bars are s.e. of the mean of the ratios for three to nine animals per point.

cut into three pieces, one containing the stained endplates, and the residual radioactivity of each piece was assayed by γ counting. The specific endplate radioactivity was determined by subtracting the radioactivity bound to the muscle pieces without endplates, from the activity in the piece containing the endplates on a per weight basis. We will call the specific endplate label at innervated junctions SC_i , that at denervated junctions which is due to the original receptors labelled before denervation SC_{Dor} , and that due to the total AChR population labelled any time after denervation SC_D .

Figure 1 plots the ratio of residual label, SC_{Dor}/SC_i , with time after denervation. This ratio is not significantly different from unity up to 8–10 days after denervation but becomes significantly lower thereafter. The early part of the post-denervation curve is consistent with the work of Berg and Hall⁴, who observed that the decay rate of original AChRs does not change up to 5 days after denervation. The drop in the ratio (Fig. 1) indicates an increase in degradation rate of the original junctional AChRs by 10 days after denervation.

The degradation half lives of these innervated and denervated junctional receptors, labelled at the time of denervation, was determined as shown in Fig. 2. The label remaining with time at the innervated junctions (Fig. 2a) shows a single exponential

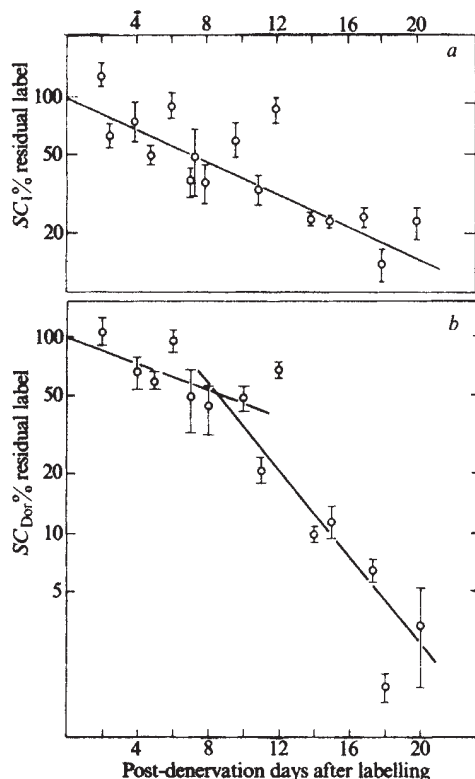


Fig. 2 Specific endplate label at different days after labelling (from all animals included in Fig. 1, plus a few additional innervated points) plotted separately for the innervated SC_i (a) and denervated SC_{Dor} (b) junctions. All curves are normalized to 100% at time of labelling, error bars are s.e. of the mean for three to nine animals per point. a, The innervated AChRs have a $t_{1/2}$ of 7.5 ± 1 days (by linear regression). b, The values for denervated original receptors can be fitted by two regression lines giving a degradation $t_{1/2}$ of 8.5 ± 3 days until 9 days after denervation and one of 2.5 ± 0.5 days thereafter. (As the $t_{1/2}$ of the original denervated receptors up to 9 days after denervation is not significantly different from that of innervated AChRs, an average value of 8 days will be used for both receptors during that period.) Although a gradual change from $t_{1/2}$ of about 8 days to a faster rate can also fit the data, we will, for convenience, assume that $t_{1/2}$ changes sharply and use the following mathematical formulation in subsequent calculations to assess the fraction of original receptors remaining at any given time (t) after denervation. When $t \leq 9$ days: $f(t) = 2 \exp[-(t/8.0)]$, and when $t > 9$ days: $f(t) = 2 \exp[-[(9/8.0) + (t-9)/2.5]]$.

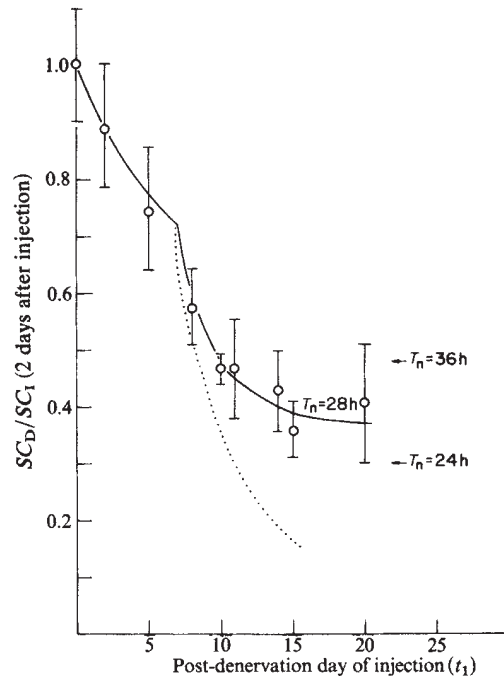


Fig. 3 Mice were injected with ^{125}I - α -bungarotoxin at different days (t_1) after denervation, thus labelling the AChRs as well as the remaining original population. The ratio of specific endplate label at innervated junctions (SC_D/SC_i) was determined 2 days after labelling. Experimental values (O) are from ref. 12 (with a few added points for confirmation). Curves were calculated on the basis of two alternative hypotheses: (1) (solid line), assuming that the junctional AChRs at denervated junctions consist of a dual population with different metabolic characteristics: the original receptors degraded as given in Fig. 2b, and new receptors, with a half life T_n . A value for T_n of 28 h gave the best fit to the data. The experimental data are within the range of values for T_n of 24–36 h (arrows) commonly associated with extrajunctional AChRs^{1-5,15,16,18,19,21,22}. (2) (dotted line), assuming a dual population as given in (1) above, but with turnover rate of the original AChRs remaining with a $t_{1/2}$ of 8.0 days throughout, and the decreasing ratios in Fig. 1 representing a progressive decrease in the overall size of the denervated endplates. This model clearly does not fit the data. Additional evidence that the endplate does not decrease in overall number of sites was obtained (as reported in the text) from the ratio (SC_D/SC_i) of 1.3 ± 0.3 for 16-day denervates 3 h after labelling.

decay (fit by linear regression) having a half time of 7.5 ± 1 days, similar to that previously reported^{1-5,8}. The residual label at the denervated junctions (Fig. 2b) due to original receptors shows (by linear regression) two exponential decays, with a half life of 8.5 ± 3 days until ~ 9 days after denervation, and of 2.5 ± 0.5 days thereafter. (From our data, we cannot determine whether the degradation rate of the original receptors changes sharply at 9 days or whether, as may be more likely, there is a gradual shift from an 8-day to a faster degradation half time.)

To test whether the drop in ratio SC_{Dor}/SC_i in Fig. 1 could be due to a decrease in the overall size of the denervated junctions as the denervated muscle atrophied, we injected four animals 16 days after denervation, waited 3 h to allow for removal of nonspecific label, removed the muscles and determined the specific endplate label by subtracting non-endplate label as described above. If there is no decrease in the number of AChRs at the denervated junction, the ratio (SC_D/SC_i) 3 h after injection should not be significantly different from unity. A value of 1.3 ± 0.3 (s.e.m.) was obtained. This is in agreement with Frank *et al.*²¹, who found no change in toxin binding at denervated endplates for up to 2.4 weeks after denervation. As electron microscope autoradiography has shown that the AChR site density remains unchanged up to 16 days after denervation⁸,

we conclude that the total size of the denervated junction did not decrease. Additional evidence that the denervated endplate size did not decrease is presented in Fig. 3. Thus, the increased degradation rate of original AChRs by 8–10 days after denervation reflects an increased turnover rate (that is, as the original receptors are degraded, they are replaced by new AChRs on a one-to-one basis).

However, the behaviour of the original AChRs cannot fully explain the behaviour of total junctional AChRs at denervated junctions. We previously¹² examined the turnover of total junctional AChRs labelled at different times after denervation, and found that these total junctional AChRs begin to show a progressive increase in their turnover rate within 2 days of denervation, and reach a turnover half time of 30 h by 15 days. Even a week after denervation, when the original junctional receptors still decay slowly (with a $t_{1/2}$ of ~8 days, as given in Fig. 2b), the average turnover half life for total junctional receptors at the denervated junction had increased considerably (to ~2 days)¹². A similar fast turnover rate was obtained for the total junctional AChRs specifically located at the postjunctional membrane of 8-day denervated neuromuscular junctions by electron microscope autoradiography⁸, indicating that the faster turnover time reported for total junctional receptors is not an artefact of the γ -counting procedure.

To reconcile the data obtained for the original receptors in Fig. 2b with those for the total junctional receptors at denervated junctions reported previously^{8,12} and in Fig. 3, we tested the assumption that after denervation, the original junctional receptors are degraded with the overall time course described in Fig. 2b, but that they are replaced by new receptors with a faster turnover half life (T_n). Based on this model we calculated the expected SC_D/SC_I for the experimental conditions of our earlier study¹². In that earlier study, the average turnover rate of total junctional receptors at denervated neuromuscular junctions was obtained over successive 2-day periods by injecting animals with ¹²⁵I- α -bungarotoxin on different days after denervation (t_1) and determining the residual junctional label 2 days later (Δt). Therefore, in that study, the contribution to SC_D due to the original receptors labelled at time t_1 and evaluated at time Δt is $f(t_1) \times 2 \exp(-\Delta t/t_{1/2})$. The $t_{1/2}$ is either 8 or 2.5 days, or a combination of both (depending on the value of t_1); and $f(t_1)$ is the fraction of original receptors remaining at the junction at time t_1 (Fig. 2b). In addition, as the absolute site density of AChRs remains constant after denervation⁸, the new receptors available for labelling at time t_1 are $[1 - f(t_1)]$, and their contribution to the specific endplate label (SC_D) at time $(t_1 + \Delta t)$ is $[1 - f(t_1)] \times 2 \exp(-\Delta t/T_n)$, where T_n is the half life of the new receptors, and is the only free parameter in this model. Figure 3 plots the experimental points for SC_D/SC_I for successive 2-day periods (Δt) as obtained in our previous study (plus new data for confirmation) and compares it with the theoretical curve (solid line) based on the above model, using $T_n = 28$ h for best fit to the data. Figure 3 shows that this model reproduces the shape of the experimental results, with the T_n well within the range reported for extrajunctional AChRs^{1-5,15,16,18,19,21,22}. Reiness *et al.*³ have reported a slow unbinding of α -bungarotoxin from AChRs *in vitro*. If we assume a similar unbinding rate ($t_{1/2}$ of 13 days) in our system *in vivo*, and include this value in the above model, the shape of the theoretical curve does not fit the experimental data. Therefore, if unbinding of toxin is occurring in our system, it must be at a considerably slower rate than that seen by Reiness *et al.*³. Another model (dotted curve) which assumes a decrease in size of the denervated junction also does not fit the data.

We conclude that the denervated neuromuscular junction contains a dual population of junctional receptors with distinct metabolic characteristics. The first consists of junctional AChRs which are present at the time of denervation and start with a $t_{1/2}$ of about 8 days and eventually (by about 9 days) lose their metabolic stability. The second consists of new receptors, which have the same metabolic characteristics as extrajunctional receptors and replace (on a one-to-one basis) the original receptors as these are degraded. Earlier studies have already

shown that the metabolic stability and high-density clustering of junctional AChRs are not linked either in development or after denervation^{8-10,23,24}. The nerve seems to have a different role in determining these two properties of junctional AChRs. Initially it is required to induce the high-density AChR localization on the postjunctional membrane. Once established, however, this localization is maintained and can even be regenerated in the absence of the nerve²⁵⁻²⁷. The present study, on the other hand, shows that a continued presence of the nerve (or some nerve-related activity) is necessary for junctional receptors both to acquire and subsequently maintain metabolic stability, even in mature muscle.

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Sexual dimorphism in a photoreceptor

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The recent observation of fluorescence emission from photoreceptor cells in flies¹ offers a new opportunity to map the distribution of the various spectral types in a highly organized retinal mosaic. Previous studies had led to the conclusion that in each ommatidium the two central receptor cells are quite distinct from their six neighbours in terms of anatomical projection^{2,3}, visual pigment and physiology⁴. We show here that in a restricted, well defined region of the male eye only, one of these central cells resembles its six neighbours not only in having the same visual pigment and physiological properties, but also in sending its axon to the same neuropile. A clue to the function of this sexually dimorphic retinal organization is given by the fact that these male-specific cells dominate the frontal-dorsal part of the retina (fovea) which is used by the male to track females in aerobatic chases^{5,6}. We suggest that the male fovea, already known to drive sex-specific higher-order neurones⁷, has sacrificed colour vision for other more vital kinds of information processing such as improving quantum catch and possibly also contrast transfer or movement detection.

Each ommatidium in the compound eye of the housefly (*Musca domestica*) contains eight photoreceptors which are

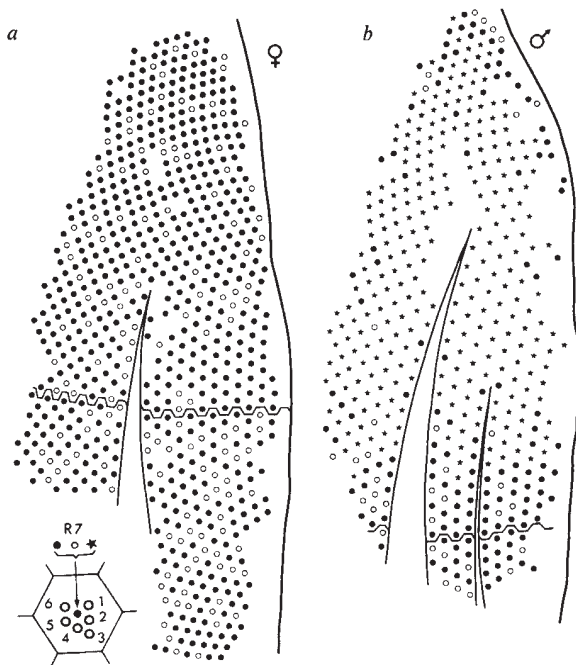


Fig. 1 Inset, arrangement and numbering of the rhabdomeres in a fly ommatidium (distal cross-section of a ventral ommatidium). Rhabdomere R8 is a proximal extension of R7. The inset indicates in brackets the three symbols used for R7 in the adjoining maps. *a*, *b*, Maps of the frontal part of the right retina showing the distribution of the three types of R7 receptor cells in the compound eye of a female (*a*) and male (*b*) housefly (*M. domestica*, white eye). Each dot or star represents the distal tip of rhabdomere R7 (see inset) as seen *in vivo* in each ommatidium, using epifluorescence microscopy (blue excitation) combined with optical neutralization of the cornea¹ (water immersion objective $\times 25/0.65$). Empty spaces represent ommatidia in which the fluorescence colour of R7 could not be clearly distinguished. ●, Green-fluorescing R7s (UV + blue-sensitive cells⁴); ○, non-fluorescing R7s (pure UV-sensitive cells⁴); asterisks, red-fluorescing R7s (dual peak, UV + blue-green-sensitive cells, see Fig. 2). The zig-zag line represents the equator of the eye, that is, a (virtual) line of dorso-ventral symmetry, about which the R1–6 rhabdomeric pattern (see inset) is inverted. About 70% green-fluorescing and 30% non-fluorescing R7s make up the female retina (*a*) and the ventral part of the male retina (*b*). The red-fluorescing R7s are present in the frontal-dorsal region of the male eye exclusively (*b*). Note how they gradually invade the retina above the equator. These maps (each of which encompasses approximately one-fifth of the fly retina) were reconstructed from 35(*a*) and 38(*b*) colour photographs with overlapping borders. Although such maps are most conveniently made in white-eyed flies, the situation seems to be qualitatively similar in wild type.

usually classified as six large peripheral cells, R1–6 surrounding two smaller central cells, R7 and R8 (see Fig. 1, inset). The photoreceptive segments (rhabdomeres) of the latter are arranged one above the other to form a single light-guiding structure³. As recognized by Cajal², the axons of R1–6 cells project only as far as the first optic ganglion (the lamina) whereas the axons of both R7 and R8 pass through this neuropile and eventually synapse first in the second optic ganglion (the medulla). A similar retinal dichotomy of long and short visual fibres seems to be a general feature of diurnal compound eyes.

The lamina is organized into optic cartridges each of which normally receives axons from six R1–6 cells 'looking' in the same direction of space, but belonging to different ommatidia^{8,9}. The signal summation which occurs within each cartridge¹⁰ necessarily increases the signal-to-noise ratio, and hence the sensitivity of these visual channels whose information is relayed to the medulla by second-order monopolar neurones^{2,3,9,11}. As the individual properties of the single receptors are buried in this summation process, it is not surprising that the six cells feeding

each cartridge all have the same (dual-peak) spectral sensitivity^{12,13}.

In contrast to the homogeneity of the R1–6 population throughout the retina, the population of central cells, R7 and R8, is highly heterogeneous. Three classes of receptor cells R7 have been found using *in vivo* epifluorescence microscopy. While R1–6 cells always fluoresce red under blue excitation, R7 rhabdomeres exhibit three distinct colours: red, green and black (that is, nonfluorescing), depending on the ommatidium¹.

We have screened large regions of the retina by rotating the fly stepwise on a precision goniometer under the immersion objective of the epifluorescence microscope, and constructed precise maps of the distribution of the R7 types from photographic series (Fig. 1). The most striking conclusion from these maps is that females do not possess any red-fluorescing R7 and that these are exclusively encountered in the frontal-dorsal part of the male eye where they account for between 85 and 95% of all R7s (asterisks in Fig. 1*b*).

This sex-specific retinal architecture stimulated a closer investigation of the properties of the red-fluorescing R7s. As their fluorescence colour was similar to that of R1–6 we thought they would have the same photopigment, and hence spectral sensitivity, as their six neighbours. Intracellular recordings and stainings revealed that the spectral sensitivities were almost indistinguishable (Fig. 2), a finding corroborated by microspectrophotometry of individual R7 rhabdomeres (ref. 14). Taken together, these two methods suggest that the red-fluorescing R7s have inherited not only the R1–6 rhodopsin, responsible for the peak near the 480 nm, but also apparently the UV-sensitizing pigment¹³, responsible for the second peak near 360 nm (Fig. 2). The two other types of R7 have spectral sensitivities quite different from those of R1–6 cells⁴.

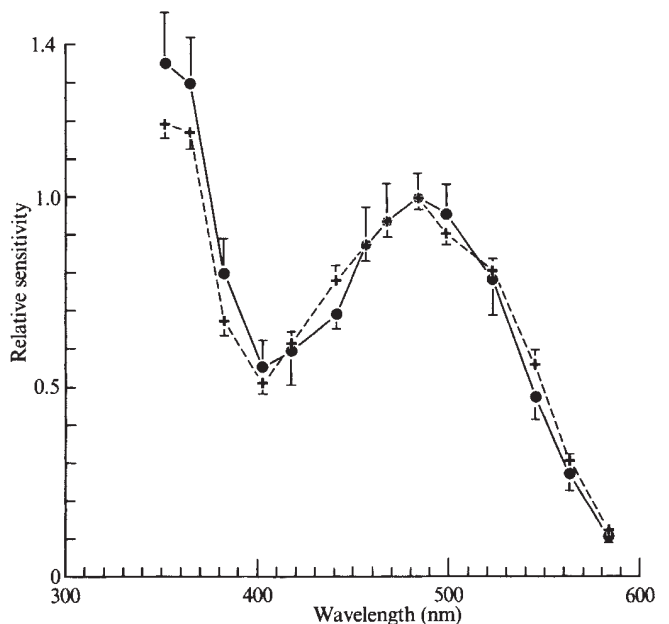


Fig. 2 Mean spectral sensitivity of red-fluorescing, male-specific R7 cells (●, $n = 12$) compared with that of peripheral cells R1–6 (+, $n = 45$). Both curves are normalized to the peak at ~ 480 nm. Vertical bars indicate the standard errors of the means. Intracellular recordings were made with Lucifer yellow¹⁷-filled glass microelectrodes ($R = 300\text{--}700\text{ M}\Omega$) (for technical details see ref. 4). Although adjustment of the direction of the electrode track and control of the depth of penetration below the cornea allowed us to aim at the desired (frontal-dorsal) retinal region, only subsequent *in vivo* recovery of the dye-injected cells¹⁵ could ascertain that the R7s recorded were indeed from a retinal region dominated by red-fluorescing R7s (asterisks in Fig. 1*b*). In contrast to the two other types of R7 of the retina⁴ (Fig. 1), the male-specific R7 cells seem to have a spectral sensitivity similar to that of R1–6 cells. *M. domestica*, white eye.

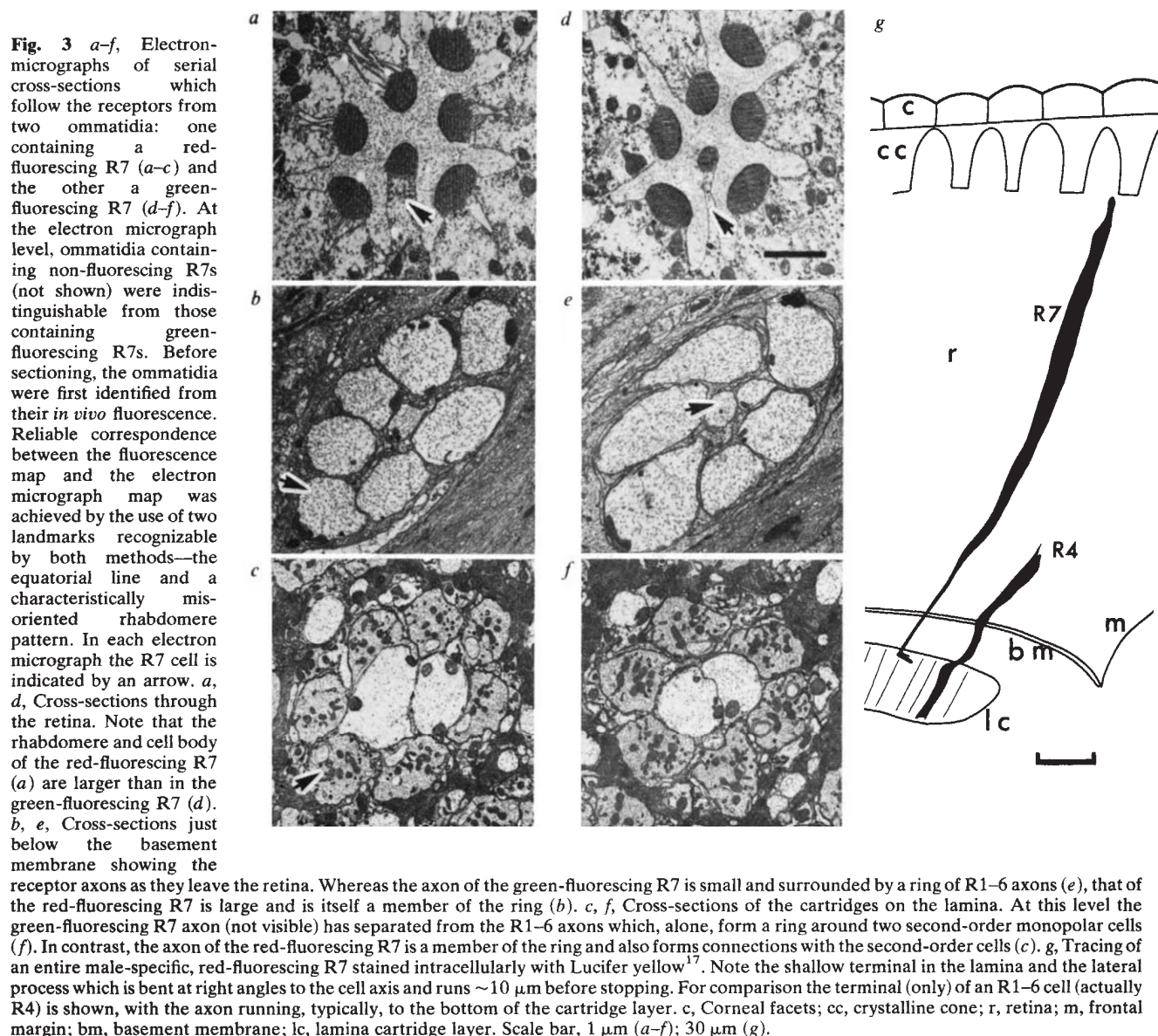
Electrophysiologically the responses of the male-specific R7s are so similar to those of R1–6 cells that it proved impossible to tell them apart during recording and only *in vivo* recovery of blindly injected cells¹⁵ revealed that we had recorded from them.

These R7 cells resemble R1–6 cells even at the ultrastructural level. Thus, electron micrographs showed that, compared with other R7 cells, the red-fluorescing ones have larger cell bodies and axons, and also wider rhabdomeres (Fig. 3)—all features which approximate to the situation in R1–6 cells. Most surprisingly, electron microscope serial sectioning established that, like R1–6 cells, the axons of these male-specific R7 cells also form connections with second-order neurones in the lamina (Fig. 3c), while axon profiles from stained cells revealed that their axons also stop short in the lamina (Fig. 3g) instead of projecting to the medulla like the other R7s of the eye.

In a restricted, well defined region of the eye of male houseflies, the R7 photoreceptors thus seem to have been programmed to resemble the peripheral R1–6 photoreceptors in terms of visual pigment, electrophysiological properties and many fine details of anatomy. Curiously, the underlying R8 photoreceptors in this region go only halfway in this respect. They apparently contain the same visual pigment as R1–6 but remain anatomically distinct, still retaining the long visual fibre input to the medulla¹⁴.

In looking for a function for this intriguing sexual dimorphism we are attracted by the recently discovered sex-specific chasing behaviour of male flies^{5,6,16} and the sex-specific neurones found in the lobula of male flies⁷. Both the behaviour and the neurones receive their main visual input from the region of the eye (the fovea) where the red-fluorescing R7s are located. The male's chasing behaviour involves the tracking of a small, fast-moving spot (the female fly) and, in visual tasks requiring high temporal resolution, light quanta are as vital as at low light levels¹⁶. In this respect the participation of a seventh, spatially and spectrally matched input to the summation process at the first synaptic level could be considered an adaptation to increase further the signal-to-noise ratio (although by a small factor) in a quantum-limited task of obvious biological importance.

However, additional functions are suggested by details of the lamina terminals of the male-specific R7s, revealed by intracellular staining. Their axons do not penetrate as deeply into the cartridge layer as those of R1–6. Furthermore, just before they terminate, they produce a right-angled branch which runs ~10 µm (that is, to about the next cartridge)—always towards the frontal margin of the eye (Fig. 3g). If this lateral process mediated inter-cartridge interactions, it could subserve a peripheral form of movement detection. Alternatively, it could modify the contrast transfer properties of second-order neurones by providing lateral inhibition.



Whereas other parts of the retina possess an impressive array of colour receptors⁴, the male fovea is necessarily colour blind. We suggest that this sacrifice is made to enhance one or more of the above mentioned processes (quantum catch, contrast transfer and movement detection), all of which can be seen as advantageous for the exacting task in which the male fovea seems to be mainly involved.

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Direct transfer of a lysosomal enzyme from lymphoid cells to deficient fibroblasts

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Fibroblasts grown *in vitro* can secrete lysosomal enzymes into the culture medium which can then be taken up by endocytosis into other fibroblasts^{1–4}. Such cells have been used as a source of enzyme for replacement therapy in the mucopolysaccharidoses^{5–7}, a group of related storage diseases resulting from inherited deficiencies in specific lysosomal enzymes, although the mechanism of action is unclear. Because lymphoid cells can interact directly with fibroblasts^{8,9} and transfer DNA to them¹⁰, we examined the possibility that a lysosomal enzyme could be transferred from normal lymphocytes to deficient fibroblasts by a mechanism other than secretion followed by endocytosis. We report here that fibroblasts from a patient with mucopolysaccharidosis type VII acquired β -glucuronidase (EC 3.2.1.31) activity as a result of direct cell-to-cell contact with lymphocytes.

Monolayer cultures of β -glucuronidase-deficient fibroblasts (GM 151 cells) contained no detectable enzyme activity, even at high cell density. After co-culture with lymphocytes, β -glucuronidase was detected in all the GM 151 cells examined (Fig. 1a). Direct measurement of enzyme activity acquired by the fibroblasts after interaction was not possible, because between 1 and 10% of the added lymphocytes adhered firmly to them even after repeated washing (Fig. 1b). Quantitative cytochemical assay in single GM 151 cells was possible, however, because the lymphocyte-fibroblast association was readily disrupted by trypsin.

The β -glucuronidase activity acquired by GM 151 cells increased rapidly during the first 24 h of co-culture, and decreased over the next several days (Fig. 2). In eight other separate experiments, maximum levels of between 29.9 and 48.1 nmol per h per 10^6 cells (mean 40.7 ± 2.1 s.e.) were acquired after 24 h.

The cytochemical assay accurately measured β -glucuronidase activity, as shown in Fig. 3. Note that the activity acquired by GM 151 cells (40.7 nmol per h per 10^6 cells) was above that in fibroblasts from two (clinically normal) people heterozygous for the β -glucuronidase allele, GM 1850 and GM 2074 (27.1 and 12.2 nmol per h per 10^6 cells, respectively), and also greater than that required for full correction of the metabolic deficiency in mucopolysaccharidosis type VII (ref. 11).

The β -glucuronidase activity acquired by 10^5 GM 151 cells was directly proportional to the number of lymphocytes added during 24 h of co-culture, up to 10^7 lymphocytes per culture dish (see Table 1). This optimum number of cells was therefore used for subsequent co-culture.

The persistence of acquired β -glucuronidase activity was examined. If, after the initial 24 h of co-culture, the non-adherent lymphocytes were replaced by fresh medium for a further 24 h, the β -glucuronidase activity decreased from an initial 40.7 nmol per h per 10^6 cells to 25.8, the same as that in fibroblasts incubated in the continued presence of lymphocytes (Fig. 2). Furthermore, the addition of 10^7 fresh lymphocytes after the initial 24 h of co-culture produced only a small further

Table 1 The effect of lymphocyte concentration on the appearance of β -glucuronidase activity after 24 h of co-culture with 10^5 GM 151 cells

Lymphocytes added per culture dish	β -Glucuronidase activity in GM 151 cells (nmol per h per 10^6 cells)
0	0
3×10^5	0
1×10^6	10.1
3×10^6	15.7
1×10^7	48.1
3×10^7	38.9

Monolayers of GM 151 cells were grown directly on 35-mm culture dishes and the co-culture initiated by the addition of the washed lymphocytes resuspended in 1 ml of Eagle's minimal essential medium (MEM), supplemented with fetal calf serum (FCS). After 24 h of co-culture, the non-adherent lymphocytes were removed and the adherent cells detached with 0.25% trypsin as described in Fig. 2 legend. After incubation with naphthol AS-BI- β -glucuronide and post-coupling with fast blue RR, quantitative cytochemistry of individual GM 151 cells was performed. The arithmetic means were calculated and converted to β -glucuronidase activity expressed as nmol per h per 10^6 GM 151 cells, as shown in Fig. 3.

increase, to 33.1 nmol per h per 10^6 cells. The β -glucuronidase activity acquired by GM 151 cells during an initial 24 h of co-culture decreased even further during the next 48 h, either in the continued presence of the lymphocytes (19.4 nmol per h per 10^6 cells) or after they had been replaced by fresh medium (14.8 nmol per h per 10^6 cells). However, when the non-adherent lymphocytes were removed after 24 h of co-culture, and the GM 151 cells re-cultured for 48 h at high cell density, β -glucuronidase activity persisted at a very high level (39.4 nmol per h per 10^6 cells). This suggests that the decrease in activity after 24 h of co-culture was due to cell division rather than enzyme degradation.

It is not clear why prolonged co-culture or fresh lymphocytes did not increase further the β -glucuronidase activity acquired by GM 151 cells. Direct assay using 4-methylumbelliferyl β -D-glucuronide (see Fig. 3 legend), showed that the 10^7 lymphocytes added initially to 10^5 GM 151 cells, contained 10–40 nmol per h of β -glucuronidase activity (1–4 nmol per h per 10^6 lymphoid cells). After 24 h of interaction, almost all the lymphocytes were recovered, while the 10^5 GM 151 cells had acquired ~4 nmol per h of enzyme. This level of acquired enzyme, 40.7 nmol per h per 10^6 cells, was over 10 times above that of the lymphocytes, and was equivalent to 10–40% of the total lymphocyte activity initially added. However, preliminary experiments showed that there was also significant increase in the β -glucuronidase activity of the recovered lymphocytes themselves as a result of direct interaction with GM 151 cells. Furthermore, lymphocytes that had already been co-cultured could induce another round of enzyme acquisition when

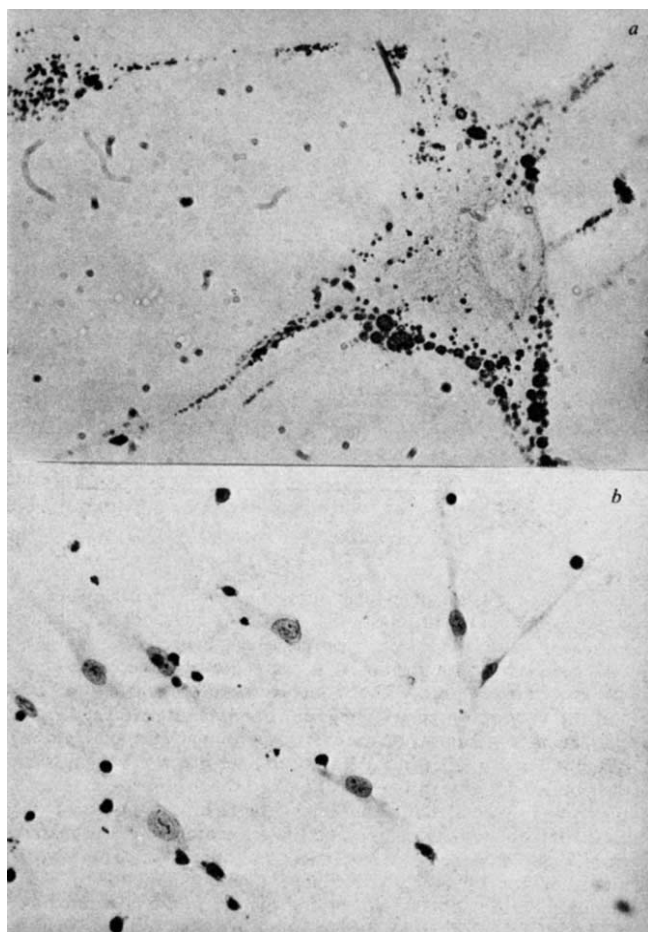


Fig. 1 Effect of lymphocytes on β -glucuronidase activity in GM 151 fibroblasts. GM 151 cells were grown to log phase (5×10^4 – 1×10^5 cells) on glass coverslips in 35-mm Petri dishes containing 1 ml MEM supplemented with 10% heat-inactivated FCS. Transformed mouse lymphocytes were prepared by incubation of mouse (CBA, 2–4-months old) spleen cell suspensions in RPMI 1640 medium containing 5% FCS, 5×10^{-5} M 2-mercaptoethanol, and $2 \mu\text{g ml}^{-1}$ Con A, at an initial cell density of 10^6 cells per ml. After 72 h in an atmosphere of 5% CO_2 in air at 37°C , the cultures consisted of a relatively homogeneous population of (thymus-derived) lymphoblasts with high intracellular levels of β -glucuronidase²⁸. The cells were collected by centrifugation and resuspended in MEM at 10^7 cells per ml. The co-culture was initiated by adding 1 ml of the lymphocyte suspension to the fibroblast monolayer. After 24 h the lymphocytes were removed and the adherent cells washed twice with 1 ml of phosphate-buffered saline (PBS). Cell numbers were determined by direct counting, using a haemocytometer. *a*, β -glucuronidase activity was measured as follows. Adherent fibroblasts were incubated with 1 ml of substrate ($100 \mu\text{g ml}^{-1}$ naphthol AS-BI- β -D-glucuronide in 0.1 M acetate buffer, pH 4.5). After 3 h at 37°C , the dishes were washed with PBS and 1.0 ml of freshly prepared fast blue RR (1 mg ml^{-1} in 0.01 M KP, pH 7.4) was added for 10 min at 4°C . The dishes were washed with PBS, methanol, then distilled water and air dried. The coverslips were removed, fixed on to slides and mounted with Polymount for visual examination and photography. GM 151 cells cultured alone did not stain in these conditions. The accumulation of enzyme is seen in densely staining vesicular organelles which look like lysosomes. $\times 500$. *b*, Adherent cells were fixed in methanol, washed with distilled water and air dried. The coverslips were removed, fixed on to slides and stained with Giemsa. The lymphocytes are smaller and more densely stained. Occasionally lymphoid cells were seen within GM 151 cells, but there was no sign of significant phagocytosis. $\times 175$.

subsequently co-cultured for 24 h with a second monolayer of GM 151 cells. Thus the β -glucuronidase activity of the lymphoblast population was not exhausted by interaction, and the fibroblasts themselves seemed to limit acquisition of activity.

Quantities of β -glucuronidase secreted by cultured cells were determined by direct assay of the supernatant media using 4-methylumbelliferyl β -D-glucuronide as a substrate. Lymphocyte culture medium and medium from lymphocyte-GM 151 co-cultures both contained less than 5 nmol per h per ml of β -glucuronidase after 24 h. Incubation of GM 151 cells

with these media for 24 h did not result in any detectable intracellular enzyme activity. The presence of a precursor to β -glucuronidase, which becomes enzymatically active after uptake by GM 151 cells, is therefore unlikely. This is consistent with the absence of such a precursor for this enzyme in murine macrophages¹². The addition to GM 151 cells of partially purified β -glucuronidase from mouse 3T3 fibroblasts (S. Diment and M. F. Dean, unpublished data) also at 5 nmol per h per ml, was similarly without effect. Moreover, after the addition of cell-free enzyme extracts derived from 10^7 lymphocytes, GM 151 cells acquired barely detectable activity.

Endocytosis of released enzyme was further examined using mannose-6-phosphate, a competitive inhibitor of receptor-mediated uptake of several lysosomal hydrolases, including β -glucuronidase^{13–15}. At 1 mM, it markedly inhibited endocytosis of exogenous enzyme prepared from 3T3 cells, but had no effect on the acquisition of β -glucuronidase by direct interaction with lymphocytes (Table 2). To produce comparable levels of enzyme activity in GM 151 cells, it was necessary to add much more exogenous β -glucuronidase derived from 3T3 cells than that present in 10^7 lymphocytes.

Concanavalin A (Con A)-mediated uptake¹⁶ of enzyme secreted by the lymphocytes also cannot account for the acquisition of β -glucuronidase activity in GM 151 cells during co-culture, because the addition of Con A ($2 \mu\text{g ml}^{-1}$), either by itself, with the culture media, or with enzyme prepared from lymphocytes, had no promoting effect on β -glucuronidase activity in GM 151 cells. The effect of α -methylmannoside (α -MM) was next examined as it inhibits Con A-mediated phagocytosis of lysosomal enzymes¹⁷. Con A-transformed lymphocytes were washed in 100 mM α -MM and then co-cultured with GM 151 cells for 24 h in the presence of 25 mM α -MM. The level of β -glucuronidase acquired by GM 151 cells was almost identical to that of control cultures. Furthermore, co-culture with lymphocytes transformed by bacterial lipopolysaccharide similarly resulted in the appearance of enzyme activity in GM 151 cells. High levels of β -glucuronidase were also acquired when fibroblasts were co-cultured with cells not exposed to mitogens, such as human peripheral blood lymphocyte/monocyte preparations and monocyte-depleted mouse spleen cells.

Cells from patients with mucopolysaccharidosis type VII produce an inactive protein which cross-reacts with antibody prepared against active enzyme^{18,19}. It is unlikely, therefore, that any factor secreted by the lymphocytes^{20–23} led to the active expression of a previously repressed structural gene.

These experiments suggest that enzyme acquisition by GM 151 cells did not involve secretion of β -glucuronidase by lymphocytes and its subsequent uptake by GM 151 cells. To test

Table 2 β -glucuronidase activity in GM 151 cells: comparison of direct cell-to-cell contact with endocytosis of exogenous enzyme

Additions to 10^5 GM 151 cells	β -glucuronidase activity in GM 151 cells (nmol per h per 10^6 cells)		
	Direct contact	Millipore* chambers	Mannose-6-P (1 mM)
10^7 lymphocytes (15)	29.9	0	39.1
Exogenous 3T3 enzyme (200)	49.9	24.1	6.1

GM 151 cells were grown to log phase (1×10^6 cells) in 35-mm culture dishes; 1 ml of medium containing 10^7 lymphocytes or partially purified enzyme was added, and the cultures continued for 24 h. Values in parentheses indicate the β -glucuronidase activity added (total nmol per h per culture dish) in the form of intact lymphocytes or purified enzyme. These values were determined by direct biochemical assay using 4-methylumbelliferyl β -D-glucuronide substrate. β -glucuronidase activity was measured cytochemically in single GM 151 cells and the mean values converted to nmol per h per 10^6 cells. GM 151 cells cultured alone had no detectable activity in these conditions.

* Millipore filters (0.22- μm pore diameter) were fitted to 20-mm diameter glass rings. The chambers, open at the top and raised on three polythene legs (2 mm high, 4 mm diameter), were placed on top of growing GM 151 monolayers, and lymphocytes or enzyme were added to the chambers. In the reverse type of co-culture, the fibroblasts were first grown on the filter inside the chamber, and the lymphocytes then placed outside in the dish. The results were identical in both culture conditions.

whether direct cell-to-cell contact was necessary, both types of cell were cultured in the same dish but were separated by Millipore filters. In these conditions, GM 151 cells did not acquire any measurable β -glucuronidase activity (Table 2). We demonstrated that free β -glucuronidase could diffuse through the Millipore membrane and be taken up by GM 151 cells on the

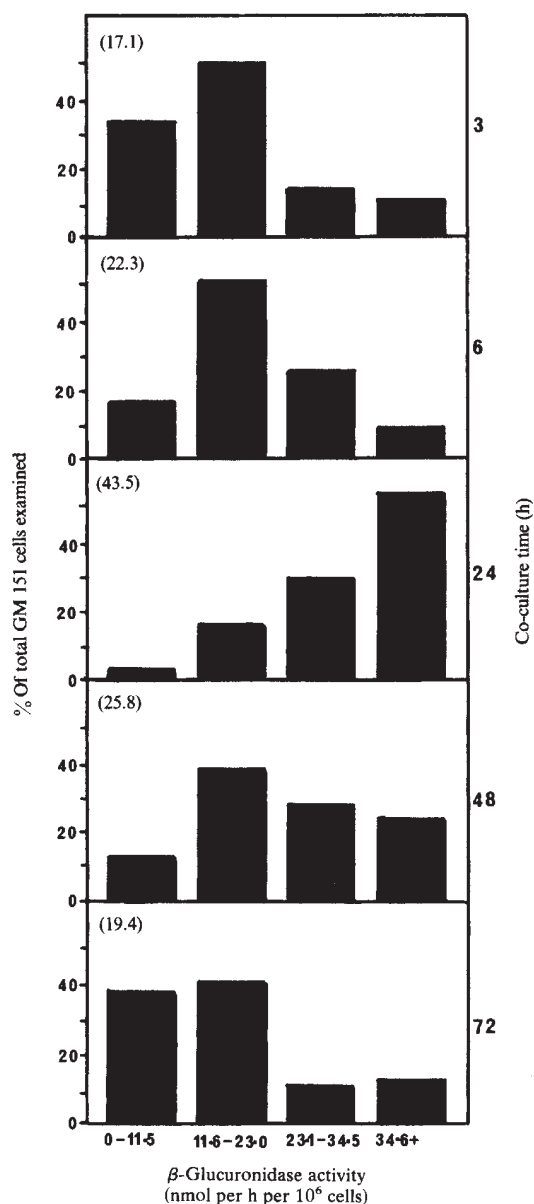


Fig. 2 Changes in β -glucuronidase activity in GM 151 cells during co-culture with lymphocytes. Monolayers of 10^5 GM 151 cells were grown directly in 35-mm culture dishes and the co-culture initiated by the addition of 10^7 lymphocytes. At the end of the co-culture periods (3–72 h), non-adherent lymphocytes were removed with PBS (37°C), and the adherent cells trypsinized (0.25% trypsin for 10 min at 37°C). After neutralization with serum, the cells were centrifuged, washed with PBS, and incubated with naphthol AS-BI- β -D-glucuronide substrate for 3 h at 37°C. The cells were centrifuged, again washed with PBS, coupled with fast blue RR, washed again and resuspended in PBS. Aliquots of the mixed cell suspension were air dried on slides, then washed in methanol and water, and re-dried and mounted. GM 151 cells were readily distinguishable by their shape and larger size. The absorption densities (at 600 nm) of 30–100 individual fibroblasts were determined on a Vickers microdensitometer M-85 using a B5 mask. Results are shown as the proportion of GM 151 cells measured within a designated range of activity, which was calculated from the absorption densities using the standard curve shown in Fig. 3. Numbers in parentheses are the arithmetic means of the single-cell β -glucuronidase activities, expressed as nmol per h per 10^6 cells. Note the gaussian distribution of β -glucuronidase activity throughout the co-culture period, rather than two distinct populations of fibroblasts, one positive and the other negative for enzyme.

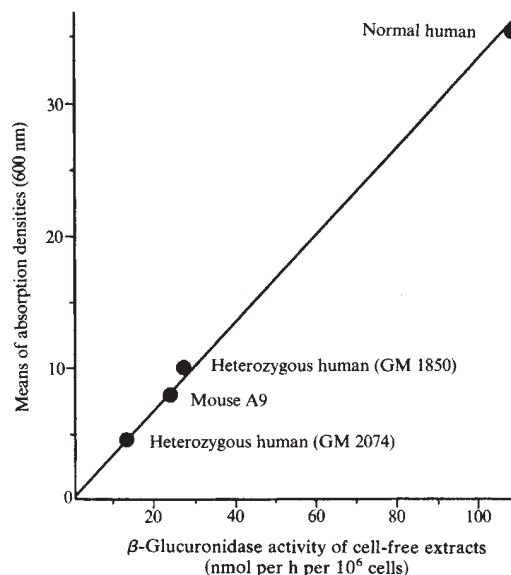


Fig. 3 Correlation of cytochemical and biochemical assays of β -glucuronidase in fibroblast cells. Mouse A9 cells, normal human fibroblasts and fibroblasts from two β -glucuronidase heterozygotes, GM 1850 and GM 2074, were grown directly on Petri dishes to late log phase. The monolayers were washed with PBS and the cells removed with trypsin. For quantitative single-cell cytochemistry, aliquots of the cell suspensions were analysed as described in Fig. 2 legend. All the cell lines had normal gaussian distributions of absorption densities, and the arithmetic means of these were calculated. For direct biochemical assay of β -glucuronidase activity, frozen and thawed extracts were prepared from aliquots of the same suspensions of fibroblasts. These were incubated for 1 h at 37°C in 2 mM 4-methylumbelliferyl β -D-glucuronide in 0.1 M acetate buffer, pH 4.5 in a final volume of 0.25 ml. One ml of 0.4 M glycine buffer, pH 10.4, was added and the fluorescence at 448 nm measured on a Locarte fluorimeter (excitation 368 nm). β -glucuronidase activity is expressed as nmol per h per 10^6 fibroblast cells used to prepare the cell-free enzyme extract. From the linear relationship between the means of the single-cell cytochemical measurements and the direct biochemical assays of β -glucuronidase in extracts of disrupted cells, a value of 2.9 nmol per h per 10^6 cells per unit density was obtained. This was used to calculate β -glucuronidase activity acquired as a result of co-culture with lymphocytes, expressed as nmol per h per 10^6 GM 151 cells.

other side by placing partially purified enzyme from 3T3 fibroblasts inside the chambers. After 24 h, enzyme uptake was 50% of that observed when the enzyme was added directly to the medium, possibly because of some adsorption of enzyme to the membrane or reduced diffusion through it. When lymphocytes and enzyme were placed in the chambers together, the uptake was again 50% of controls, showing that the lymphocytes themselves had no influence on diffusion of enzyme.

Identical results were obtained with fibroblasts from a different patient with mucopolysaccharidosis type VII (GM 121). These findings indicate that the acquisition of β -glucuronidase activity was not due to the release of enzyme from lymphoid cells followed by its uptake by deficient fibroblasts. Thus although the precise nature of the interaction between lymphocytes and fibroblasts is unknown, direct cell-to-cell contact seems to be essential for the acquisition of enzyme activity. The present results might also explain the naturally occurring exchanges of β -glucuronidase observed in tetraparental mice²⁴, the presence of α -mannosidase activity in chimaeric mannosidosis calves²⁵, and perhaps also the transient clinical benefits following leukocyte infusion in Sanfilippo's²⁶ and Hunter's²⁷ syndromes.

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Diazepam induces mitotic arrest at prometaphase by inhibiting centriolar separation

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We report here that we have found an accumulation of condensed mitotic figures in cultures of various human haematopoietic cell lines treated with diazepam ($40\text{--}80\ \mu\text{g ml}^{-1}$). The mode of mitosis arresting activity of diazepam was analysed with cultured human fibroblasts. Unlike conventional anti-mitotic drugs, diazepam did not affect the microtubular integrity at the concentrations used. Instead, it seems to block mitosis by inhibiting the deviation of centrioles in the prometaphase.

The different appearances of demecolcine-arrested and diazepam-arrested mitoses are seen in Fig. 1, which shows cytocentrifuged cell smears from cultures of the human hairy cell leukaemia line, JOK-1¹. The mitotic arrest seems to be complete as the same frequency of mitotic figures was seen after 6 and 18 h in parallel cultures treated with $5\ \mu\text{g ml}^{-1}$ demecolcine or $50\ \mu\text{g ml}^{-1}$ diazepam.

The mechanism of the diazepam-induced mitotic arrest was further studied with cultured human fibroblasts. The mitotic apparatus of untreated fibroblasts in metaphase showed a typical spindle-shaped structure in indirect tubulin-specific fluorescence (IIF) (Fig. 2a) and in double-fluorescence staining for DNA, a bright plate of condensed chromosomes in the middle of the spindle (Fig. 2b). Non-dividing fibroblasts in the same cultures showed a fibrillar tubulin-specific staining throughout

the cytoplasmic domain (Fig. 3a). Fibroblasts cultured in the presence of demecolcine ($0.2\ \mu\text{g ml}^{-1}$) for 6 h showed only a diffuse tubulin-specific fluorescence both in interphase cells (Fig. 3b) and in mitotic cells (Fig. 2c) with dispersed chromosomes (Fig. 2d).

A distinct accumulation of mitotic cells (up to 25–30%) with condensed chromosomes was seen in fibroblast cultures treated with $80\ \mu\text{g ml}^{-1}$ diazepam for 12 h. When the mitotic cells were stained for tubulin by the IIF technique, an asterisk-like array of microtubules was seen directed towards the centre of the condensed chromosomal plate (Fig. 2e, f). A distinct pair of centrioles could be seen in the centre of the microtubule asterisk (Fig. 2e, inset). Cells in telophase stage, with a completed chromosome separation, were not observed in diazepam-treated cultures, and the appearance of microtubules in non-

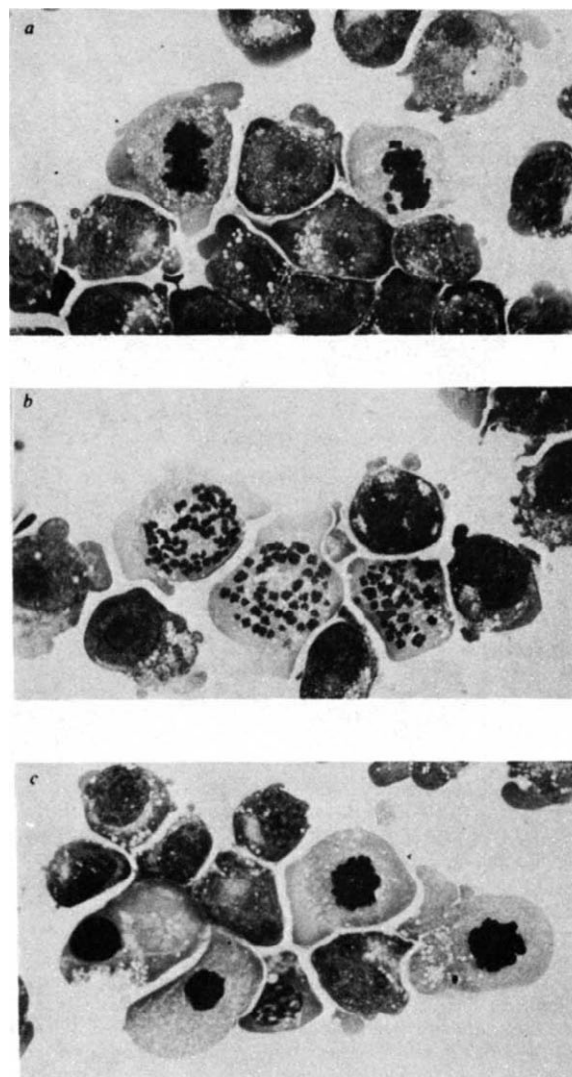


Fig. 1 May-Grünwald-Giemsa stained smears of JOK-1 cells cultivated in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS). a, Control culture; b, culture treated with $0.5\ \mu\text{g ml}^{-1}$ demecolcine for 6 h; c, culture treated with $50\ \mu\text{g ml}^{-1}$ diazepam for 6 h. Diazepam (Orion) was dissolved in dimethyl sulphoxide (DMSO). The diazepam-treated cultures contained 0.04–0.08% DMSO. DMSO at these concentrations did not measurably interfere with cell growth. The frequency of mitotic figures at 6 and 18 h after initiation of cultures were 1.5 and 2.5% in control cultures, 6 and 23% in demecolcine-treated cultures, and 11.5 and 25.5% in diazepam-treated cultures. $\times 800$.

mitotic cells was unaltered (Fig. 3). This is different from the effect seen with conventional anti-mitotic drugs (for example, demecolcine, vinblastine sulphate), which arrest cultured cells at mitosis by disrupting the microtubules of the mitotic apparatus^{2,3}, as is also indicated by the diffuse staining in IIF with anti-tubulin antibodies³ and dispersion of chromosomes.

Clark and Ryan recently reported that tranquillizers of the benzodiazepine group inhibit the proliferation of 3T3 fibroblasts in pre-S stage⁴. Our findings indicate that diazepam specifically introduces a block in the prometaphase by a unique mechanism: without affecting the microtubular integrity the

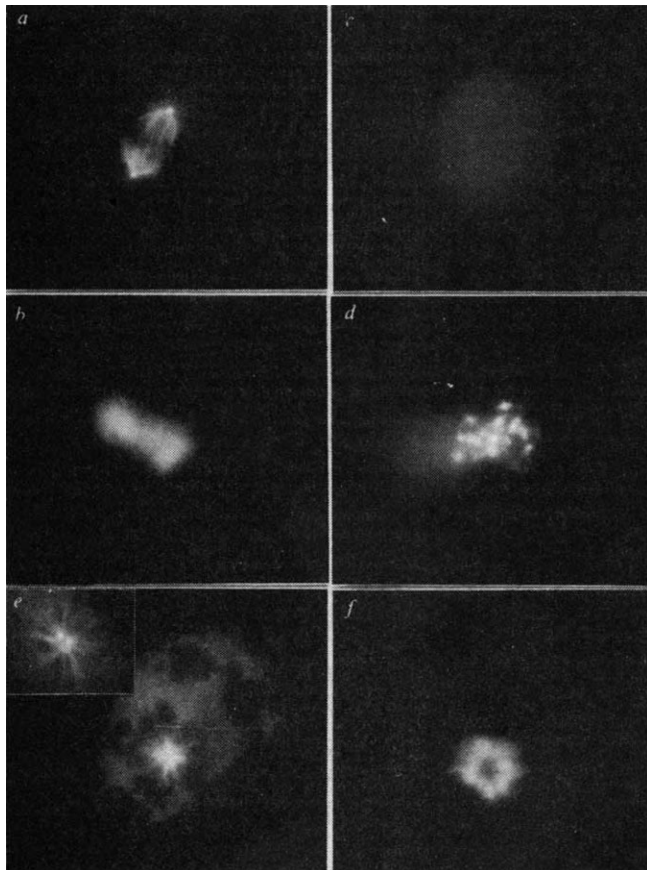


Fig. 2 Human embryonal fibroblasts were cultured in RPMI-1640 medium plus 10% FCS. For IIF microscopy the cells, cultured on small glass coverslips, were fixed in methanol for 10 min at -20°C . Microtubules were visualized using rabbit antibodies against tubulin from calf brain^{6,7}. Fluorescein isothiocyanate-coupled anti-rabbit IgG (Cappel) was used as a second antibody. Chromosomes were visualized by a double-fluorescence technique using the Hoechst no. 33258 fluorochrome⁸. In untreated fibroblasts at metaphase a bright spindle-like tubulin-specific fluorescence is seen in *a*. Note the bright spots, representing centrioles, at the poles of the spindle. In double-fluorescence with the Hoechst fluorochrome (*b*) the chromosomes were seen as a bright plate in the middle of the spindle. When fibroblasts were cultured in the presence of $0.2\text{ }\mu\text{g ml}^{-1}$ demecolcine for 6 h, rounded mitotic cells accumulated in the cultures. In such cells only a diffuse tubulin-specific staining (*c*) and scattered chromosomal figures (*d*) were seen. When human fibroblasts were cultured in the presence of diazepam ($80\text{ }\mu\text{g ml}^{-1}$ for 12 h), IIF microscopy with anti-tubulin antibodies revealed an asterisk-like mitotic apparatus (*e*) directed towards the centre of the condensed chromosomal plate as seen in double fluorescence microscopy with the DNA-fluorochrome (*f*). The centrioles, visualized as bright spots with tubulin-antibodies, were seen as a distinct doublet in the centre of the microtubule asterisk (*e*, inset). $\times 680$.

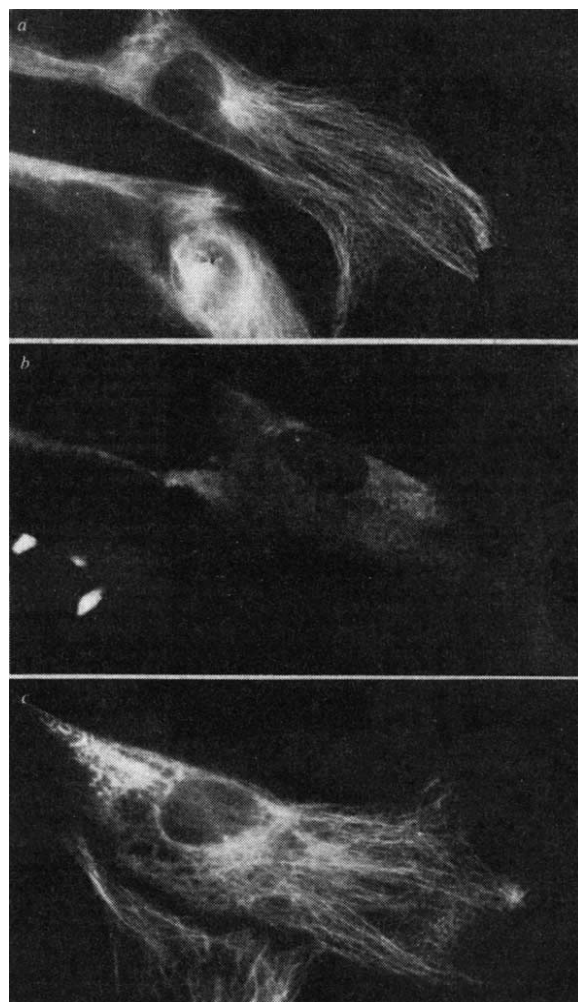


Fig. 3 In normal non-dividing cultured fibroblasts microtubules were visualized by the IIF technique as fibrillar cytoplasmic arrays throughout the whole cytoplasmic domain (*a*). After addition of demecolcine only a diffuse cytoplasmic tubulin-specific fluorescence was seen in the treated cells (*b*). In fibroblasts cultured in the presence of diazepam ($80\text{ }\mu\text{g ml}^{-1}$), normal fibrillar arrays of microtubules were seen (*c*). $\times 400$.

centriolar separation is inhibited, giving rise to an asterisk-like mitotic apparatus. Interestingly, such a variant mitotic apparatus has also recently been described to occur at low frequency during spontaneous mitosis of PTK₂ cells⁵.

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Intermediate filaments in 3T3 cells collapse after intracellular injection of a monoclonal anti-intermediate filament antibody

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The function of intermediate filaments within cells is unclear, largely because there are no known reagents or physical conditions which specifically affect their organization (see ref. 1 for review). I report here that the intracellular injection of a monoclonal IgG1 antibody specific for intermediate filaments² causes the rapid and reversible collapse of all intermediate filaments within 3T3 mouse fibroblasts while leaving the organization of microfilament bundles and microtubules apparently unaltered. Direct observation of cells undergoing intermediate filament collapse indicates that such collapse occurs without obvious effects on cell morphology or motility.

Monoclonal antibody against an intermediate filament antigen (IFA)² was purified from ascites fluid raised in *nu/nu* mice on a column of *Staphylococcus* A-Sepharose (Pharmacia), concentrated by vacuum dialysis, dialysed into injection buffer (114 mM KCl, 20 mM NaCl, 3 mM MgCl₂, 3 mM NaH₂PO₄, pH 7 with KOH (ref. 3)) and spun at 100,000g for 30 min before use. About 2×10^{-14} l (ref. 4) of antibody at a concentration of 3 mg ml⁻¹ was injected into 3T3 (clone A31) cells growing on glass coverslips for 20–48 h. During and after injection, cells were maintained at 37°C until they were fixed after various periods of time with methanol for 10 min at -20°C. The cells were then stained sequentially with fluorescein-coupled goat anti-mouse immunoglobulin (G-anti-MIg-F) to visualize microinjected antibodies and by rabbit anti-tubulin⁵, rabbit anti-cytoplasmic myosin⁶ or more monoclonal anti-IFA antibodies followed by rhodamine-coupled goat anti-rabbit immunoglobulin (G-anti-RIg-Rd) or G-anti-MIg-Rd. Appropriate control experiments indicated that all reagents used were specific (data not shown). After washing, the coverslips were mounted in glycerol and viewed with a Zeiss Universal microscope equipped with phase contrast and epifluorescence optics.

When fixed within 1 h of injection and stained with G-anti-MIg-F, many injected cells were seen to have their intermediate filaments collapsed within a bundle near or around the cell's nucleus. When such cells were exposed to more anti-IFA antibody followed by G-anti-MIg-Rd, no intermediate filament staining was observed (Fig. 1) outside the collapsed bundle. On exposure of these to either anti-tubulin or anti-cytoplasmic myosin antibodies, followed by G-anti-RIg-Rd, most microtubules and microfilament bundles were unaffected by the collapse of intermediate filaments (Fig. 2). Moreover, the collapsed intermediate filaments did not label with either the anti-tubulin or anti-myosin antibodies (Fig. 2). Cells injected with tetramethylrhodamine-coupled bovine serum albumin or antibody directed against cytoplasmic myosin⁶ never showed collapsed intermediate filaments, excluding the possibility that filament collapse was a response to injection.

The collapse of intermediate filaments by injected anti-IFA antibody was reversible, as demonstrated by time-course experiments such as those outlined in Table 1. Filament collapse was most marked at 1–3 h post-injection when more than 70%

Table 1 Time course of collapse of intermediate filaments by injected anti-IFA antibody

Time after injection (h)	Cells recovered	Cells with collapsed intermediate filaments
1	90	79 (88)
3	89	63 (71)
6	94	45 (48)
16	68	16 (24)

100 cells were injected on each coverslip and after staining, the entire coverslip was scanned. The fact that less than 100% of the cells were 'recovered' reflects both cell death due to injection trauma and the inability to recognize cells due either to reduction of staining level to near background, or interfering flaws in mounting. Numbers in parentheses are the percentage of recovered cells found to be collapsed.

of injected cells had collapsed intermediate filaments; with time the percentage decreased and the extent of filament collapse in individual cells diminished until, at 16 h after injection, many cells had fully extended intermediate filaments, although these were still decorated with anti-IFA antibody. Autophagy of injected antibody⁷ might have reduced the intracellular concentration of antibody to a level insufficient to maintain filament collapse.

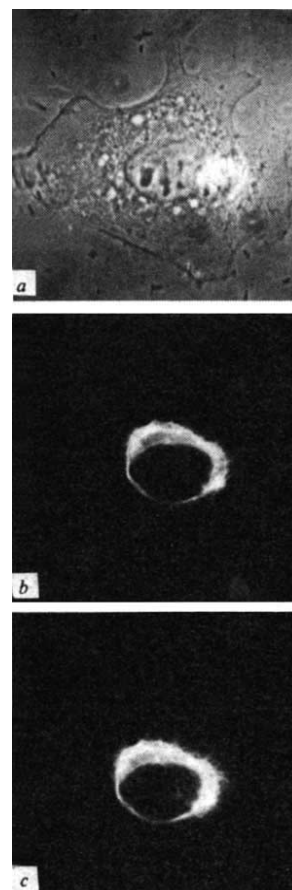


Fig. 1 Phase (a) and immunofluorescence of 3T3 cell injected with anti-IFA 1 h previously, fixed and then stained sequentially with G-anti-MIg-F, anti-IFA and G-anti-MIg-Rd. Intermediate filaments are collapsed around the nucleus (b, fluorescein optics) and secondary staining with anti-IFA shows no intermediate filaments outside the collapsed bundle (c, rhodamine optics). Cells were injected in HEPES-buffered minimal Eagle's media (MEM) + 10% fetal calf sera (FCS) + 50 µg ml⁻¹ gentamycin and then returned to bicarbonate-buffered MEM + 10% FCS and kept in 8% CO₂ at 37°C until fixation. $\times 700$.

To study the effects of intermediate filament collapse on cell morphology and movement, purified tetramethylrhodamine-conjugated anti-IFA (anti-IFA-Rd) was injected into 3T3 cells and the cells were followed by time-lapse video using a Zeiss inverted microscope equipped with phase contrast and epifluorescence optics coupled to a silicon-intensified target video camera (RT Laboratories) and video recording system. Cells were examined using epifluorescence optics at the end of the observation period. Injected anti-IFA antibody spread rapidly throughout the cell and decorated intermediate filaments. Most cells retained their pre-injection morphologies, although a small proportion underwent a rapid but reversible contraction; as this type of acute shape change was just as frequently observed after injection of buffer alone, it was probably due to the injection process itself. Most injected cells showed intermediate filament collapse within 1–3 h and the morphologies and movements of the majority of such cells could not be distinguished from uninjected cells in the same field. An example of intermediate filament collapse in two injected cells is shown in Fig. 3. Because all the cells were continuously moving and changing shape, I cannot exclude the possibility that the collapse of intermediate filaments was associated with subtle changes in cell shape or movement.

The collapse of intermediate filaments induced by anti-IFA antibody was not restricted to 3T3 cells. The intermediate filaments of baby hamster kidney-21 (BHK-21) cells and secondary chick heart fibroblasts also underwent intermediate filament collapse after antibody injection and retained substantially unaltered morphologies. Very large cells often failed to show antibody-induced filament collapse. Whether this is the result of a failure to inject sufficient quantities of the antibody or is due to a true difference in the intermediate filaments of such cells is unclear.

The mechanism of anti-IFA antibody-induced collapse of intermediate filaments is unknown. The antibody has been shown to bind to vimentin, the major protein in intermediate filaments in 3T3 cells². However, the simple binding of an antibody to filaments within living cells does not invariably change the organization of those filaments. For example, rabbit anti-cytoplasmic myosin antibodies injected into 3T3 cells decorated microfilament bundles but had no appreciable effect on the distribution of either microfilament bundles or intermediate filaments within the cells (data not shown). Moreover, various monoclonal antibodies which react with tonofilaments in PtK₂ cells⁸ decorate these filaments after injection but do not change their distribution; anti-IFA antibodies, on the other hand, collapse tonofilaments in PtK₂ cells after injection⁹.

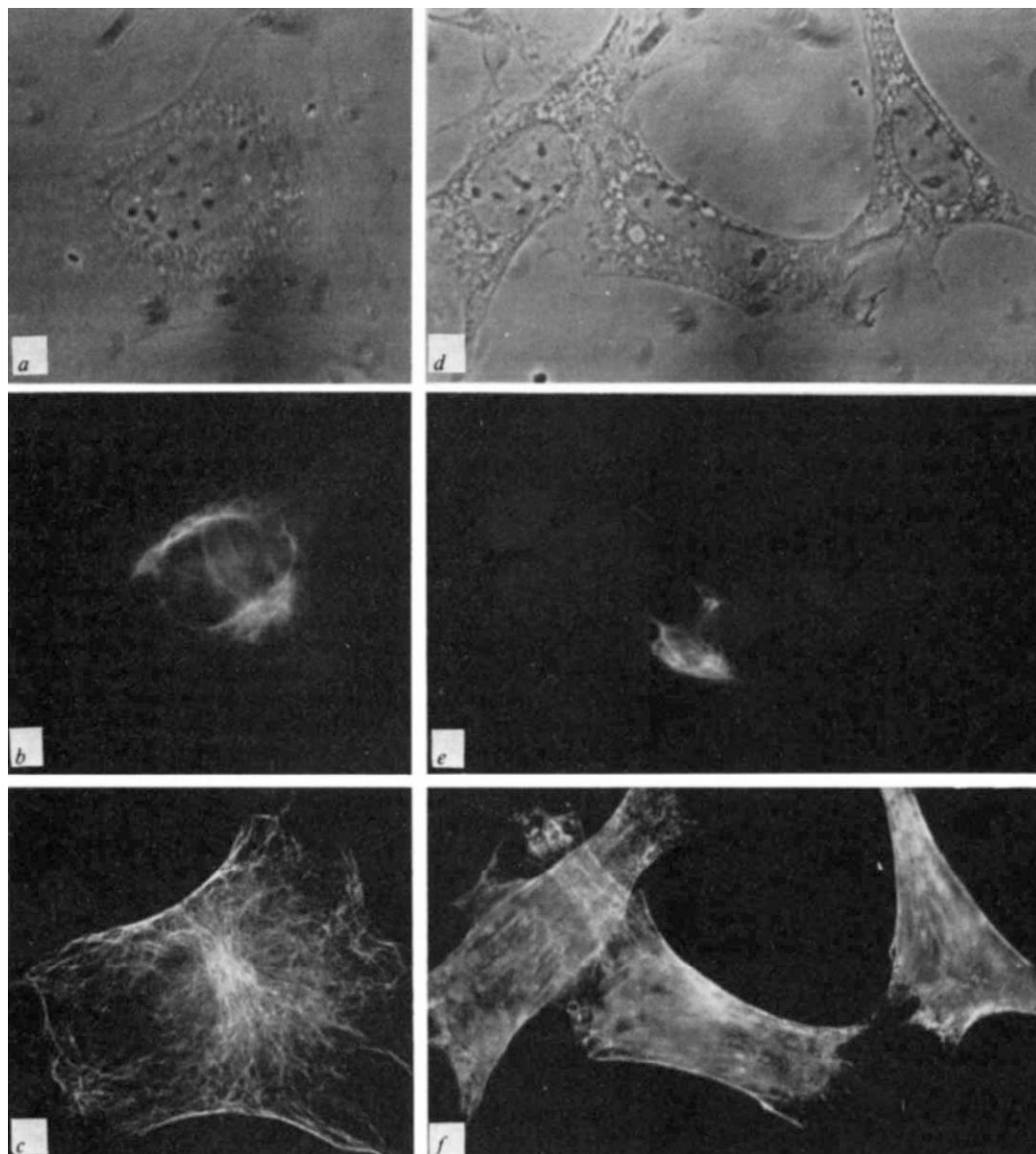
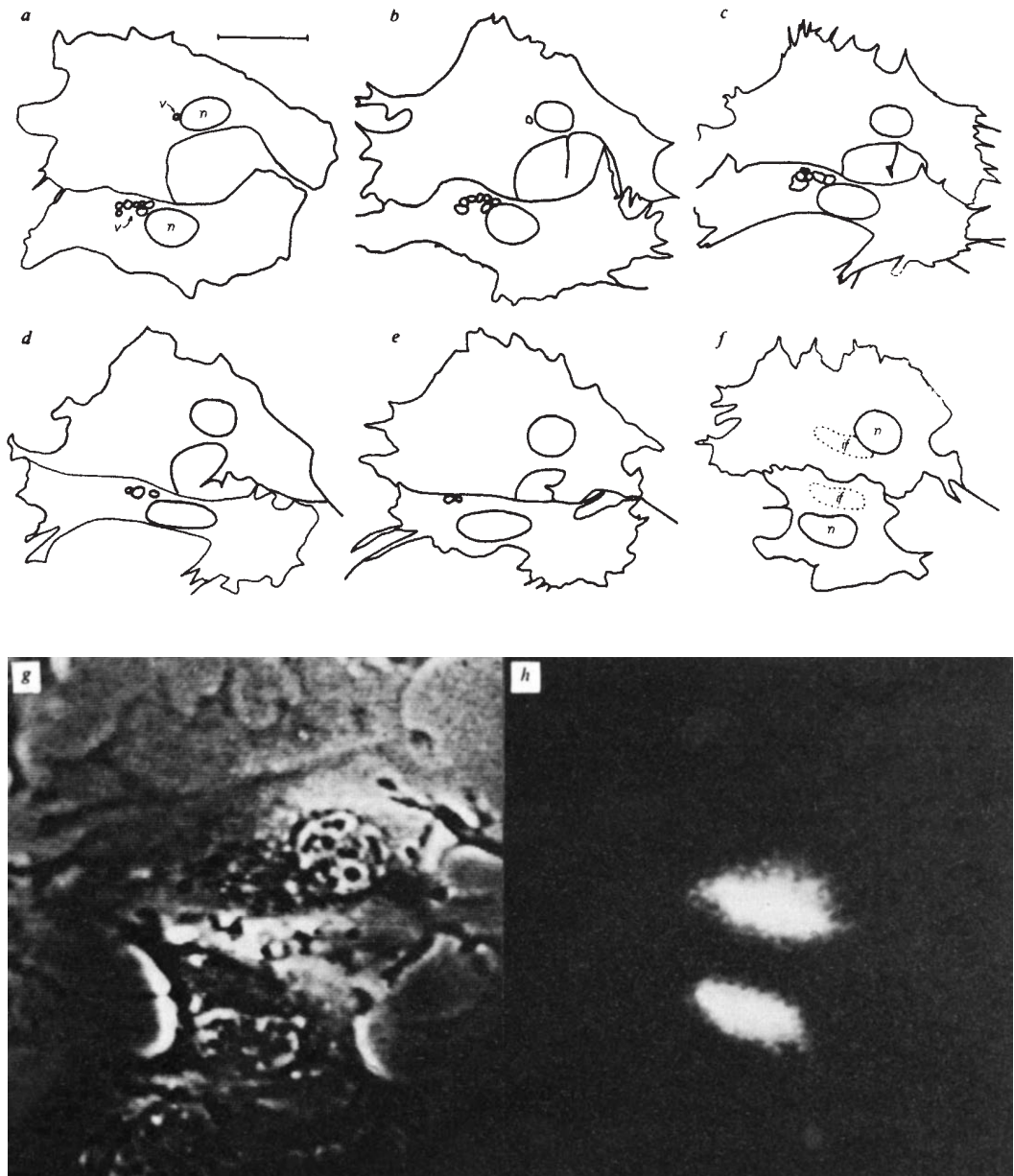


Fig. 2 Phase (a, d) and immunofluorescence of anti-IFA injected 3T3 cells (1–1.5 h before fixation) that have been stained sequentially with G-anti-MIg-F, rabbit anti-tubulin (a, b, c) or rabbit anti-myosin (d, e, f) and G-anti-RIg-Rd. Both rabbit anti-tubulin and myosin and anti-rabbit-Ig-Rd were diluted into purified mouse immunoglobulin (final concentration $\sim 2 \text{ mg ml}^{-1}$) to block possible cross-reactivity with G-anti-MIg-F. G-anti-RIg-Rd had been pre-adsorbed with mouse immunoglobulin. Intermediate filaments (b, e, fluorescein optics) are collapsed by injected anti-IFA but microtubule and microfilament bundle organization appear unaltered (c, f, rhodamine optics). $\times 1,060$.

Fig. 3 Two cells injected with anti-IFA-Rd were followed continuously by time-lapse video. Outlines of the cells were traced from the video images before (a) and 2.5 min (b), 17 min (c), 36.5 min (d), 59.5 min (e), and 76 min (f) after injection. The cells were viewed under rhodamine optics 76 min after injection, revealing their collapsed intermediate filaments (region defined by dotted boundary and 'if' in f). The phase contrast (g) and rhodamine optics images (h) of the cells 76 min after injection as photographed from the video monitor are also shown. The cells' nuclei 'n' are labelled (a, f) and vacuoles 'v' (in a), which subsequently disappear. Similar shape changes to those seen in these two injected cells are frequently seen in normal, uninjected 3T3 cells over this period of time. Cells were maintained at 37 °C in HEPES-buffered MEM+10% FCS + 50 $\mu\text{g ml}^{-1}$ gentamycin throughout. To minimize evaporation of media the culture dish was overlaid with paraffin oil. Cells survived and divided apparently normally in these conditions for longer than 24 h. Scale bar, 50 μm .



Increasing indirect evidence suggests that intermediate filaments and microtubules are functionally and perhaps physically associated with each other¹⁰. The effects of microtubule depolymerization, whether by specific drugs or physical conditions, results in the eventual collapse of intermediate filaments, not unlike that seen after injection of anti-IFA, although with a much slower time course¹. The results presented here indicate that whereas the extended organization of intermediate filaments may well depend on microtubules, the converse is clearly not the case. Apparently, intermediate filaments can be specifically withdrawn from the cytoskeletal network of the cell without obvious effects on the organization of microfilament bundles or most microtubules, or on gross cell morphology or movement. These findings suggest that intermediate filaments may have a more subtle role than previously believed.

While this manuscript was in preparation, similar results were reported in abstract form by Lin and Feramisco¹¹ using a monoclonal IgM antibody directed against a 95,000 molecular weight protein associated with intermediate filaments.

I thank members of the MRC Neuroimmunology Project, especially Dr R. Pruss for anti-IFA antibody and Drs M. Raff, M. Noble, E. B. Lane and S. Bevan, for their stimulating suggestions and encouragement, Dr T. Preston for the use of his micromanipulator, and Drs P. Sheterline and J. Fallon for gifts of anti-tubulin antisera and anti-cytoplasmic myosin antibodies respectively. I am a Muscular Dystrophy Association of America postdoctoral fellow.

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Regulation of human platelet myosin light chain kinase by the catalytic subunit of cyclic AMP-dependent protein kinase

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A rise in the concentration of cyclic AMP inhibits secretion in human platelets¹⁻³. In mammalian cells the mechanism of action of cyclic AMP involves activation of a protein kinase and results in protein phosphorylation⁴. It has previously been shown that myosin light chain kinase purified from smooth muscle is a substrate for the catalytic subunit of cyclic AMP-dependent protein kinase⁵ and that phosphorylation of smooth muscle myosin kinase results in a decrease in its activity⁶. Here we present evidence that platelet myosin kinase is a substrate for the catalytic subunit of protein kinase and that phosphorylation of this myosin kinase, isolated from a non-muscle cell, decreases the activity of this enzyme. Dephosphorylation of platelet myosin kinase by a purified phosphatase⁷ restores its original activity. A decrease in myosin kinase activity in platelets would increase the relative amount of unphosphorylated myosin, which unlike phosphorylated myosin, cannot interact with actin^{8,9}. These findings suggest a mechanism by which cyclic AMP might modulate actin-myosin interaction in platelets and other non-muscle cells.

The platelet myosin kinase used was purified by affinity chromatography using a column of calmodulin-Sepharose 4B (refs 10, 11). Figure 1 shows the time course of incubating purified platelet myosin kinase, [γ -³²P]ATP and the catalytic subunit of protein kinase in the presence and absence of protein kinase inhibitor. In the presence of the catalytic subunit, ³²P was rapidly incorporated into platelet myosin kinase, whereas incubation in the presence of the protein kinase and its specific inhibitor decreased the amount of ³²P incorporation by ~90%. Incubation in the absence of protein kinase showed only minimal amounts of incorporation of ³²P after 1 h. A time course experiment carried out in the absence of protein kinase showed no evidence for autophosphorylation during earlier times (not shown).

To verify that platelet myosin kinase served as the phosphate acceptor, a sample of myosin kinase that had been incubated for 45 min with [γ -³²P]ATP and protein kinase was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The major protein peak (M_r = 100,000), corresponding to myosin kinase, coincided with the only peak of protein-bound ³²P. When PAGE was carried out in the absence of SDS, a single peak of myosin kinase activity, which contained all the ³²P, was eluted from the gel (data not shown).

The stoichiometry of phosphorylation was determined both by Millipore filtration of phosphorylated myosin kinase following precipitation with trichloroacetic acid and by quantifying the amount of protein and ³²P migrating with myosin kinase in SDS-PAGE. In four separate preparations an average of 1.2 mol of phosphate per mol of myosin kinase was incorporated. However, these results do not exclude the possibility that more than one site can be phosphorylated.

Figure 2 shows the effect of phosphorylation on platelet myosin kinase activity and is a double reciprocal plot of data derived from activation of myosin kinase over a range of calmodulin concentrations. Two effects of phosphorylation are observed. First, phosphorylation results in a threefold decrease in maximal enzymatic activity (from 0.148 to 0.054 nmol min⁻¹). Second, the concentration of calmodulin required for half-maximal enzyme activity is threefold greater for phosphorylated myosin kinase than for the unphosphorylated species (2.8×10^{-8} M compared with 0.9×10^{-8} M).

The effect of phosphorylating myosin kinase is reversible, as shown in Fig. 3, which compares the specific activity of unphosphorylated, phosphorylated and dephosphorylated platelet myosin kinase at two different concentrations of calmodulin. Figure 3 also shows an autoradiogram indicating the state of phosphorylation of the kinase before and after incubation with phosphatase. The enzyme used to dephosphorylate myosin kinase was the purified phosphatase I prepared from turkey gizzards⁷, which was free of protease activity. The ability of smooth muscle phosphatase I to dephosphorylate platelet myosin kinase and restore the higher activity of the unphosphorylated enzyme is consistent with a role for this phosphorylation in regulating the activity of myosin kinase.

Phosphorylation of the 20,000- M_r light chain of platelet myosin and other non-muscle myosins has been shown to have two important effects: (1) it is required for the actin-activation of the Mg-ATPase activity of myosin^{8,9,12-14}, and (2) it promotes the assembly of myosin filaments in physiological conditions¹⁵. The enzyme catalysing phosphorylation of platelet myosin has been purified and shown to have an absolute requirement for

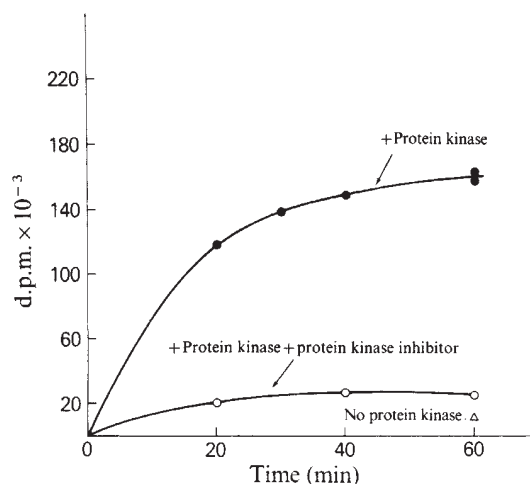


Fig. 1 Time course of phosphorylation of platelet myosin light chain kinase by the catalytic subunit of cyclic AMP-dependent protein kinase in the presence and absence of the specific inhibitor of protein kinase. Myosin light chain kinase was purified as described by D.R.H. and R.S.A.¹⁰ except that the crude extract was applied to a 20-ml column of AH-Sepharose 4B and eluted with 0.5 M NaCl, replacing the DEAE-Sepharose chromatography, and Sephacryl S-300 was used rather than S-200. The purified kinase (5×10^{-8} M) was incubated at 23 °C in 50 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 25 μ M [γ -³²P]ATP (2×10^7 c.p.m. nmol⁻¹) and 2×10^{-7} M cyclic AMP-dependent protein kinase (a gift of Dr E. H. Fischer). This was done in the presence (○) and absence (●) of the specific inhibitor of cyclic AMP-dependent protein kinase¹⁸ (a gift from Dr L. Rappaport). Time points were taken at 20, 30, 40 and 60 min, and the amount of ³²P incorporated into the myosin kinase was determined by Millipore filtration and liquid scintillation counting of the filter disks¹². A single time point was taken in the absence of protein kinase (△), showing no phosphorylation of the platelet myosin kinase. When histone IIA was phosphorylated at comparable concentrations using the same amount of protein kinase, the time course of phosphorylation was similar to that obtained with platelet myosin kinase. Phosphorylation of platelet myosin kinase was not dependent on Ca²⁺.

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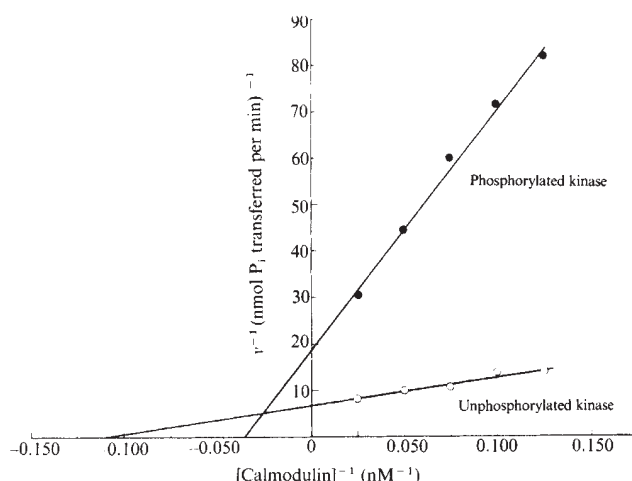


Fig. 2 Double reciprocal plot showing the effect of phosphorylation on the activity of platelet myosin light chain kinase. The myosin kinase (3×10^{-7} M) was incubated for 45 min at 23°C with 40 mM Tris-HCl (pH 7.5), 5 mM MgCl_2 and 4×10^{-7} M cyclic AMP-dependent protein kinase, in the presence (●) and absence (○) of $50 \mu\text{M}$ ATP. The resulting phosphorylated and unphosphorylated samples of myosin kinase were then each assayed over a range of calmodulin concentrations from 9×10^{-9} M to 4×10^{-8} M. Calmodulin was prepared from bovine brain by the method of Klee¹⁹. The concentration of platelet myosin kinase was 3.3×10^{-8} M. The assays were carried out for 1 min at 23°C in 50 mM Tris-HCl (pH 7.3), 10 mM MgCl_2 , 0.1 mM CaCl_2 , $100 \mu\text{M}$ [$\gamma\text{-}^{32}\text{P}$]ATP (2×10^6 c.p.m. nmol^{-1}) and $25 \mu\text{M}$ smooth muscle myosin 20,000- M_r light chain. This myosin light chain fraction was prepared as outlined previously⁵. Total assay volume was $100 \mu\text{l}$.

Ca^{2+} and the ubiquitous calcium-binding protein, calmodulin^{10,11}.

Previous work has shown that the enzyme catalysing myosin phosphorylation in smooth muscle can be phosphorylated by the catalytic subunit of cyclic AMP-dependent protein kinase⁵. Phosphorylation has a marked effect on the ability of the smooth muscle enzyme to bind calmodulin, but only a small effect on V_{max} (ref. 6). The results reported here for platelet myosin kinase differ in that phosphorylation of the platelet myosin kinase decreases both the ability of the enzyme to bind calmodulin and the activity of the enzyme when extrapolated to an infinite concentration of calmodulin (V_{max}). This difference in

the effect of phosphorylation on the kinetic parameters of the two enzymes may simply reflect the species difference between the turkey gizzard smooth muscle kinase and that isolated from human platelets. On the other hand, it is possible that the purified platelet myosin kinase, which has a molecular weight 30,000 less than the smooth muscle kinase (100,000 compared with 130,000), has undergone partial proteolysis. Such proteolysis, which does not alter the requirement of the enzyme for calmodulin or the ability to respond to phosphorylation, could be responsible for a subtle alteration in the kinetic response. Further work will be necessary to rule out this latter possibility.

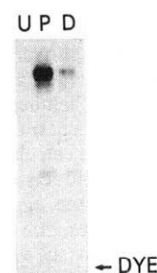
Cyclic nucleotide-mediated phosphorylation of myosin kinases may provide an important mechanism for modulating contractile activity in non-muscle cells. A rise in the intracellular concentration of cyclic AMP would result in the phosphorylation of myosin kinase and hence decrease myosin kinase activity. The net result of this decreased activity would be an inhibition of contractile activity because it would result in a relative increase in the unphosphorylated form of myosin. Indirect evidence for cyclic AMP-mediated regulation in smooth muscle cells has been provided by Kerrick *et al.*¹⁶, who showed that addition of the catalytic subunit of cyclic AMP-dependent protein kinase to chemically skinned smooth muscle fibres resulted in a decrease in both tension and the phosphorylation of smooth muscle myosin light chains.

Daniel *et al.*¹⁷ have shown that platelet secretion can be correlated with the phosphorylation of platelet myosin, indicating that secretion results from contraction of platelet actomyosin. This finding, together with data from physiological experiments suggesting that agents which increase cyclic AMP in platelets act to inhibit secretion¹⁻³, is consistent with our *in vitro* findings that phosphorylation of platelet myosin kinase by cyclic AMP-dependent protein kinase results in inhibition of myosin kinase activity. Of course, it is possible that cyclic AMP works through more than one pathway to cause a decrease in contractile activity in platelets. To confirm the physiological significance of the mechanism outlined here, future experiments must: (1) demonstrate phosphorylation of platelet myosin kinase *in vivo* in response to a rise in intracellular levels of cyclic AMP and (2) correlate this phosphorylation with inhibition of a contractile event. However, our present data suggest that phosphorylation of myosin kinase by cyclic AMP-dependent protein kinase is one mechanism by which cyclic AMP regulates contractile activity in platelets and other non-muscle cells.

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Fig. 3 The effect of dephosphorylation of myosin kinase activity. Platelet myosin kinase was incubated in the presence and absence of $1.25 \mu\text{M}$ catalytic subunit of cyclic AMP-dependent protein kinase. Also present in both tubes were 50 mM Tris-HCl (pH 7.3), 10 mM MgCl_2 , 0.1 mM CaCl_2 and $20 \mu\text{M}$ [$\gamma\text{-}^{32}\text{P}$]ATP. After 30 min at 23°C , 20 mM EDTA was added to each sample to inhibit further phosphorylation. The sample with the protein kinase was then divided into two parts, to one half of which was added 6×10^{-9} M purified smooth muscle phosphatase I^7 (a gift of Dr Mary Pato). The resulting three tubes (U=no protein kinase, no phosphatase; P=+protein kinase, no phosphatase; D=+protein kinase, +phosphatase) were incubated for 15 min at 23°C before assay. The left-hand side is an autoradiogram showing the state of phosphorylation of the phosphorylated (P) and dephosphorylated (D) samples. There is no evidence for autophosphorylation of the myosin kinase in the unphosphorylated (U) sample. The dye front is indicated. In wells P and D, halfway between the ^{32}P band due to the myosin kinase and the dye, is a light band which is probably due to autophosphorylation of the catalytic subunit of protein kinase. The samples were assayed at the two calmodulin concentrations shown, using 1×10^{-9} M platelet myosin kinase. The assays were terminated after 15 min (1×10^{-9} M calmodulin) or 8 min (3×10^{-9} M calmodulin) at 23°C , with other conditions as in Fig. 2 legend.

	Specific activity of myosin kinase ($\mu\text{mol per mg per min}$)	
	$1 \times 10^{-9}\text{M}$ Calmodulin	$3 \times 10^{-9}\text{M}$ Calmodulin
UNPHOSPHORYLATED	0.21	0.98
PHOSPHORYLATED	0.09	0.45
DEPHOSPHORYLATED	0.23	0.96



Note added in proof: Recent experiments indicate that phosphate is incorporated into two different sites in platelet myosin kinase (C.R.E. and R.S.A., unpublished), as recently reported for smooth muscle myosin kinase⁶.

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Localized gating of egg Na⁺ channels by sperm

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Eggs of many species respond to sperm with a positive shift in membrane potential, the fertilization potential¹, which in several^{2–5} but not all⁶ species mediates a block to polyspermy. How the sperm elicits the fertilization potential is not known, although it is the earliest known response of eggs to sperm, occurring within 1–2 s of mixing the gametes. In the marine worm, *Urechis caupo*, the fertilization potential results from an increase in Na⁺ permeability which is amplified during the first 15 s by a voltage-gated Ca²⁺ action potential⁷. The Na⁺ channels are gated not by voltage, but by sperm, a sperm opening only a fraction of available channels⁷. The experiments reported here indicate that the open Na⁺ channels are localized in a 'patch' near the fertilizing sperm.

Because the amplitude of the fertilization potential depends on the external Na⁺ concentration ([Na⁺])⁷, it is possible to distinguish between a localized and a uniform distribution of open Na⁺ channels if the concentration of Na⁺ at the site of fertilization is altered independently of that over the rest of the egg. The experimental apparatus, described in Fig. 1 legend, consisted of a perforated nickel screen in which the egg was seated separating two chambers containing seawater of the same or different [Na⁺]; [Na⁺] was either 470 mM (natural seawater) or 1/10th that amount. By injecting a dense sperm suspension into this chamber, then removing the egg and measuring the area to which sperm were bound, at $\times 400$ with an ocular micrometer, we calculated that 5–10% of the egg surface was exposed to the solution in the lower chamber. Because the resistance through the egg interior is small relative to that across the membrane, the egg is isopotential, and the electrode in the upper surface of the egg accurately records the fertilization potential initiated by a sperm at the opposite side of the egg. Furthermore, the lower chamber was not electrically isolated from the upper chamber, as the two chambers were connected by a low resistance pathway even with an egg seated firmly in the hole. The resistance across the open hole, measured with an ohmmeter, was about 10 k Ω , and increased by only 1–4 k Ω with an egg in the hole. These resistances are trivial compared with

that across the egg membrane (≥ 100 M Ω), and thus introduce no significant error into the electrical measurements. Sperm, however, did not cross from one chamber into the other during the experiments. Microscopic examination of 10 eggs inseminated in the lower chamber showed that sperm were bound only to that portion of the egg (easily identified by a bleb, or deformation in the egg surface coat) that had been in the lower chamber. (Supernumerary *Urechis* sperm do not unbind rapidly from the eggs after fertilization.)

The results of six combinations of 470 and 47 mM Na⁺ seawater with sperm added to one or the other chamber (Fig. 2, Table 1) show that a sperm must open a localized patch of Na⁺ channels in its vicinity. Figure 2a–d shows that the initial amplitudes of the fertilization potentials are dependent on [Na⁺] at the fertilization site regardless of the [Na⁺] bathing the rest of the egg. Figure 2e shows that average plateau potentials (see legend) are also predicted by the [Na⁺] at the site of fertilization. With approximately the upper 90% (see above) of the egg

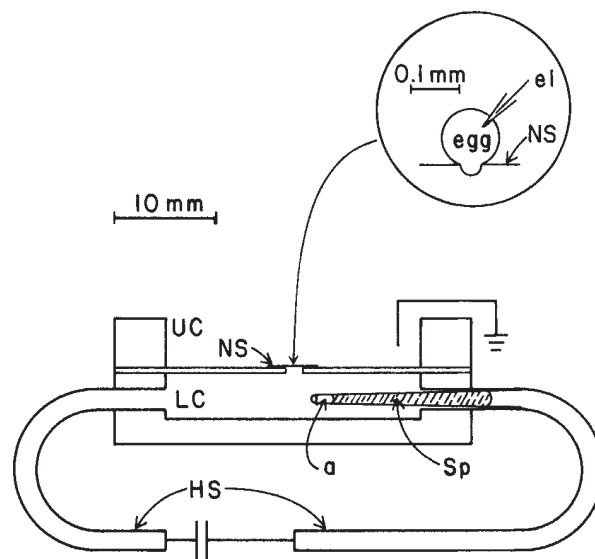


Fig. 1 Diagram of the experimental chamber modified from the original design of Robinson and Jaffe¹³. An egg (120 μ m diameter) is seated in a 45- μ m diameter hole in a nickel screen (NS; Perforated Products Inc.) separating two chambers (UC, upper chamber; LC, lower chamber) which can contain seawater of differing [Na⁺]; sperm are added to one of the two chambers. A small piece of nickel screen is glued with non-toxic Paraplast over the hole in the thin plastic barrier separating upper and lower chambers. All but one hole in the nickel screen is plugged with Paraplast, and this hole is the only opening between upper and lower chambers. The respective volumes of seawater in the upper and lower chambers are 0.7 and 2 ml. Each of the two entry ports in the lower chamber is connected by thin plastic tubing to a 10- μ l Hamilton syringe (HS). The left-hand syringe is used to create a slight suction that will seat the egg firmly in the hole. The right-hand syringe delivers sperm to the lower chamber. The sperm delivery tube contains about 5 μ l sperm suspension (Sp) prevented from prematurely entering the chamber by an air bubble (a) at the tip. (Before inserting the tube in the chamber, external sperm are killed by washing the outside of the tube in deionized water.) To inject the sperm suspension into the lower chamber, the plungers of the two syringes are arranged back to back as shown, permitting simultaneous addition and withdrawal of equal volumes so that the position of the egg in the hole is not disturbed. Eggs were observed (at $\times 100$ with a Wild stereomicroscope) to ensure they did not move in the hole. Sperm are delivered to the upper chamber dropwise from a pipette. At the beginning of an experiment, both upper and lower chambers are filled with seawater of the [Na⁺] desired in the lower chamber, the sperm delivery tube is inserted into the lower chamber (or the port is plugged if sperm are to be added from above), the egg is seated in the hole and penetrated with a microelectrode, then the solution in the upper chamber is changed, if desired, by perfusion with a simple siphon plus vacuum suction system (not shown). The solution in the upper chamber was continuously perfused whether or not its composition differed from that in the lower chamber. One-tenth [Na⁺] seawater was prepared by diluting natural seawater with Na⁺-free artificial seawater (NaCl replaced with choline-Cl). Methods for microelectrode measurements were those used by Jaffe *et al.*⁷. Methods for maintaining *Urechis* and obtaining and handling gametes are described elsewhere¹⁴.

Table 1 Average plateau potentials of the fertilization potentials obtained by varying $[Na^+]$ at the site of fertilization independently of $[Na^+]$ on the rest of the egg surface

Condition*	$\bar{x}$ (mV)	Average plateau potential s.e.m.†	(n)	P‡
470	+18.2	2.4	(5)	<0.02
470s				
47	+17.8	6.5	(5)	
470s				
47s	-14.3	4.4	(3)	>0.05
470				
47	-26.7	2.0	(3)	
47s				
470s	+18.0	3.0	(3)	<0.02
47				
470	+3.2	2.2	(5)	
47s				

See legend to Fig. 2e for method of calculation of average plateau potential. Data in the first four lines of the table are the same as in Fig. 2e.

*mM Na^+ in upper chamber

mM Na^+ in lower chamber, s indicates chamber to which sperm were added.

†Standard error of the mean (standard deviation/ $n^{1/2}$).

‡Calculated by the *t*-test, comparing the means of the two samples indicated by the brackets.

surface in low- Na^+ seawater and the lower 10%, to which sperm are added, in high- Na^+ seawater, the average fertilization potential amplitude, +17 mV, is the same as that obtained with high $[Na^+]$ bathing the entire egg. However, if sperm are added to the upper egg surface in low $[Na^+]$, the average plateau potential, -14 mV, is significantly less (see Table 1). The -14 mV potential obtained with 47s/470 and the -27 mV potential with 47/47s are not significantly different at the 95% confidence level (Table 1, s indicates the chamber to which sperm were added). In reciprocal experiments, the $[Na^+]$ in the two chambers was reversed, with the upper 90% of the egg in high $[Na^+]$ and the lower 10% in low $[Na^+]$. Again, fertilization in low $[Na^+]$ resulted in a plateau potential (+3 mV) significantly more negative than that (+18 mV) obtained with fertilization in high $[Na^+]$ (Table 1). However, a more positive potential was observed with 470/47s (+3 mV) than with 47/47s (-27 mV). This could be due to leakage of Na^+ from the upper into the lower chamber through the egg's thick (>1 μ m) surface coat. (A slight suction from upper to lower chamber is applied to hold the egg in the hole, and the solution bathing the lower surface of the egg is not renewed by perfusion.) Alternatively,

Table 2 Fertilization potential characteristics of eggs seated in the experimental apparatus compared with controls resting in the bottom of a Petri dish

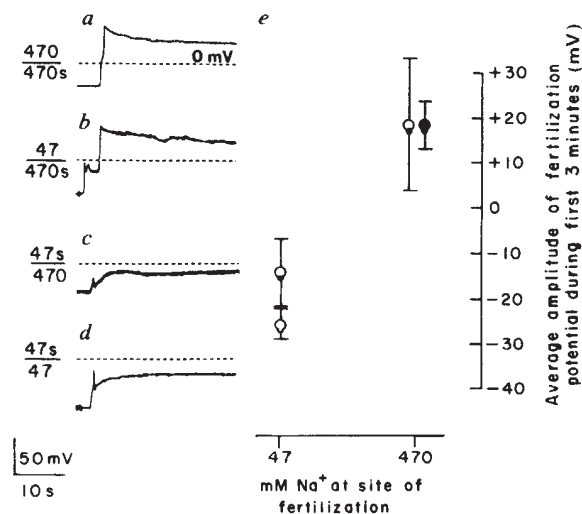
	Average plateau potential (mV)	Fertilization potential resistance (M Ω)	Unfertilized egg resistance (M Ω)
Natural seawater			
Eggs in holes	+17 $\pm$ 2.3 (5)	146 $\pm$ 23 (3)	
Controls	+25 $\pm$ 1.3 (3)	135 $\pm$ 20 (3)	548 $\pm$ 55 (3)
1/10th $[Na^+]$ seawater			
Eggs in holes	-26 $\pm$ 2.0 (3)		
Controls ⁷	-21 $\pm$ 1.6 (10)		

Average plateau potential was calculated for the first 3 min as described in Fig. 2 legend for eggs in this study; in the study of Jaffe *et al.*⁷, values were calculated for the first 5 min. Resistance measurements for the controls were obtained during the 2–3-min interval after the rise of the fertilization potentials, by passing pulses of inward current ranging from -0.2 to -3×10^{-10} A, and calculating the slope resistance of the resulting current-voltage plots, which were linear. Slope resistances for the unfertilized eggs were obtained in the same way except that the membrane potential was first set to a value (+15 to +24 mV) comparable with that during a fertilization potential, and the inward current pulses superimposed. These *I-V* plots were also linear. Resistances for the eggs in holes were determined from the voltage deflection resulting from a single inward current pulse (-1.8×10^{-10} A) at 2 min after the rise of the fertilization potential. For experiments with eggs in holes, sperm were added to the lower chamber. The values are averages $\pm$ s.e.m. (standard deviation/ $n^{1/2}$), with *n* in parentheses.

the patch of Na^+ channels opened by sperm may be large enough to include some of the egg surface in the opposite chamber.

Controls indicate that the observed 'patch' is not an artefact of the experimental conditions. First, the fertilization potentials of eggs in the experimental apparatus appear normal. The maximum amplitudes and average plateau potentials of eggs seated in holes are similar to those of control eggs resting in a grooved plastic dish (Table 2). In addition, measurements of egg membrane resistance during fertilization potentials in natural seawater showed that sperm-induced increases in egg membrane conductance ($G = 1/R$) are also similar in experimental and control eggs; in both situations, the conductance increased approximately fourfold over that in unfertilized eggs (Table 2, columns 2, 3), indicating that the opening of Na^+ channels is not significantly restricted by the experimental chamber. Second, eggs seated in the nickel screen activate normally. Activation was scored in four of the eggs that were fertilized from below in normal seawater. While the eggs remained seated in the hole, all four underwent normal germinal vesicle breakdown, although the germinal vesicle was in the upper portion of the egg, and in all four, surface coat elevation occurred around the entire egg. Three of these eggs were removed from the hole and cultured further; all formed polar bodies and cleaved.

The finding that sperm open a patch of Na^+ channels is consistent with our earlier suggestion (see above and ref. 7) that the fertilization potential may be analogous to the receptor potential plus action potential system found in many chemo- and mechanosensitive cells. The Na^+ channels are not contributed by the sperm membrane, but must pre-exist in the egg because fertilization potentials can be elicited by artificial agents (trypanin⁸) in the absence of sperm⁷. However, we do not know whether the Na^+ channels are opened by an interaction of sperm and egg membrane components before bilayer fusion (the neurotransmitter-receptor analogy) or whether fusion must occur to initiate a fertilization potential. Whether sperm-egg fusion occurs rapidly enough (within a few seconds of insemination) to initiate the fertilization potential is not known (see Discussion, ref. 7). It also remains to be determined whether the Na^+ channels are opened by a direct interaction of sperm and

**Fig. 2** Fertilization potentials obtained by varying the $[Na^+]$ at the site of fertilization independently of that over the rest of the egg surface. a-d. Original chart records showing initial portions of fertilization potentials obtained in the conditions indicated: mM Na^+ in upper chamber, s indicates chamber to which sperm were added. e. Average plateau potentials as a function of $[Na^+]$ at the site of fertilization. The values were obtained by averaging the potentials at 0.5, 1, 1.5, 2, 2.5 and 3 min after the initial rise of the fertilization potentials. *Urechis* egg fertilization potentials typically remain positive for about 7 min. The bars indicate the standard deviation of the mean (see Table 1 for the value of *n*). ●, Both chambers contain 470 mM Na^+ , sperm added from below; ○, the lower chamber contains 470 mM Na^+ and the upper chamber 47 mM Na^+ ; ●, both chambers contain 47 mM Na^+ , sperm added from below.

egg membrane components, or whether intermediate 'messengers' (for example, Ca^{2+}) are involved. Based on iontophoretic injection experiments, Cross⁹ has proposed that a sperm-induced rise in free Ca^{2+} beneath the egg plasma membrane may initiate the fertilization potential in frog eggs. Another interesting question that remains to be answered is whether the patch of open Na^+ channels is confined to the site of sperm egg membrane contact, or whether it extends laterally.

In addition to contributing to our understanding of the gating mechanism for the fertilization potential, the results reported here raise the question of the possible significance of the 'patch' for later development. In *Urechis* eggs, the plane of the first cleavage generally passes through the point of sperm entry (within 10° of this point in 70% of the eggs^{10,11}). The localized patch of open Na^+ channels may be involved in establishing this polarity¹².

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Stringency without ppGpp accumulation

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Wild-type *Escherichia coli* deprived of a required amino acid shows a stringent response involving various alterations in the cell's normal physiology, including the arrest of net synthesis of stable (ribosomal and transfer) RNA, lipids, phospholipids and peptidoglycans¹. The stringent response is found in cells of the Rel^+ (stringent) but not Rel^- (relaxed) phenotype. The intracellular concentration of guanosine 5'-diphosphate-3'-diphosphate (ppGpp) and guanosine 5'-triphosphate-3'-diphosphate (pppGpp) increases dramatically in amino acid-deprived Rel^+ cells due to the enzymatic activity of the *relA* gene product², which also stimulates ppGpp synthesis *in vitro*³. The correlation between stringent response and increase in the intracellular concentration of ppGpp has been interpreted as evidence that this nucleotide has a causal role in stringency¹. However, as recent findings show that the correlation does not always hold, ppGpp cannot be considered to act as a unique effector molecule and the idea of a causal role for ppGpp in stringent control has become questionable^{4,5}. Here we describe experiments that cast further doubt on the proposed biological role of this nucleotide, as they show that stringent control can occur in the absence of ppGpp accumulation.

The genome and the physiology of *Salmonella typhimurium* are closely related to those of *E. coli*, and it can be safely assumed that the principal aspects of stringent control do not differ substantially in the two species. In fact, in *S. typhimurium* the net synthesis of stable RNA has been shown to be under stringent control and *relA* mutants similar to those found in *E. coli* have been isolated⁶. In addition, ribosomes prepared from *relA*⁺ *S. typhimurium* produce ppGpp *in vitro*⁷. We were therefore not surprised to find that amino acid deprivation of cultures of *relA*⁺ *S. typhimurium* auxotrophs normally causes the accumulation of ppGpp to the same high levels observed in *E. coli* in the same conditions. However, one notable exception is shown in Fig. 1. When strain TA1001 (*hisT*, *hisD* 2655) was deprived of its sole amino acid requirement, histidine, the level of ppGpp did not rise. Even with repeated experiments there was only an occasional modest (at most twofold) increase in the ppGpp level which did not last for >5 min. When such an increase was observed, it was also seen in control experiments where the culture was filtered and resuspended in medium containing histidine. We therefore attribute this nonreproducible, slight and transient increase in ppGpp to the filtration procedure itself and not to amino acid deprivation. In these experiments the level of pppGpp never rose to values detectable by our methods.

Strain TA1001 was obtained by co-transducing the markers *aroC*⁺, *hisT*1514 and *purF*⁺ into strain TA997 (*aroC*5, *purF*145, *hisD*2655) (R. Cortese, personal communication). Figure 1 shows that when strain TA997 was deprived of histidine, both ppGpp and pppGpp rose to high levels, as expected for a *relA*⁺ strain. The basal level of ppGpp was similar in both strains.

Strain TA1001 has no amino acid requirements other than histidine. It can, however, be deprived of serine by treatment with the amino acid analogue, serine hydroxamate. After such

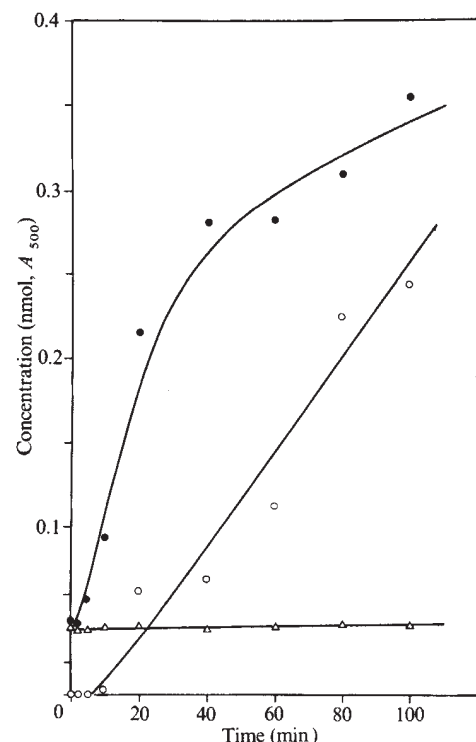


Fig. 1 Intracellular concentration of ppGpp and pppGpp in strains TA1001 and TA997 deprived of histidine. Bacteria were grown at 37 °C in low-phosphate medium⁴ containing 10^{-3} M H_2HPO_4 to a concentration of 2×10^8 cells per ml, $100 \mu\text{Ci ml}^{-1}$ $^{32}\text{H}_3\text{PO}_4$ were added and after one doubling the cells were filtered on Millipore filters and resuspended in medium lacking histidine, but containing $100 \mu\text{Ci ml}^{-1}$ $^{32}\text{H}_3\text{PO}_4$. Samples were taken and analysed as described elsewhere⁴. Δ, ppGpp, strain TA1001; ●, ppGpp, strain TA997; ○, pppGpp, strain TA997.

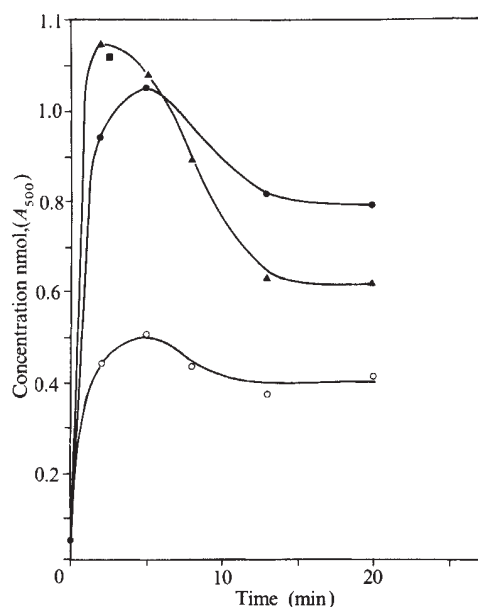


Fig. 2 Intracellular concentration of ppGpp in strain TA1001 and auxotrophic derivatives of TA1001 deprived of different amino acids. The same procedure was used as for Fig. 1. ●, Strain TA1001 deprived of serine by addition of $400 \mu\text{g ml}^{-1}$ serine hydroxamate; ○, strain PD11 deprived of threonine; ▲, strain PD12 deprived of arginine. Strains PD11 (*hisD2655*, *hisT1514*, *thrA557::Tn10*) and PD12 (*hisD2655*, *hisT1514*, *argB1837::Tn10*) were obtained by transduction into TA1001.

treatment, very high levels of ppGpp and pppGpp are produced (see Fig. 2). Strain TA1001 can also be transduced by phage P22 carrying *S. typhimurium* DNA with the transposon *Tn10* inserted in and inactivating a gene belonging to an amino acid biosynthetic pathway. Figure 2 also shows that two such derivatives of TA1001, one requiring arginine and the other threonine, also produce ppGpp and pppGpp when deprived, respectively, of these two amino acids. Thus, strain TA1001 is not intrinsically incapable of raising its ppGpp and pppGpp

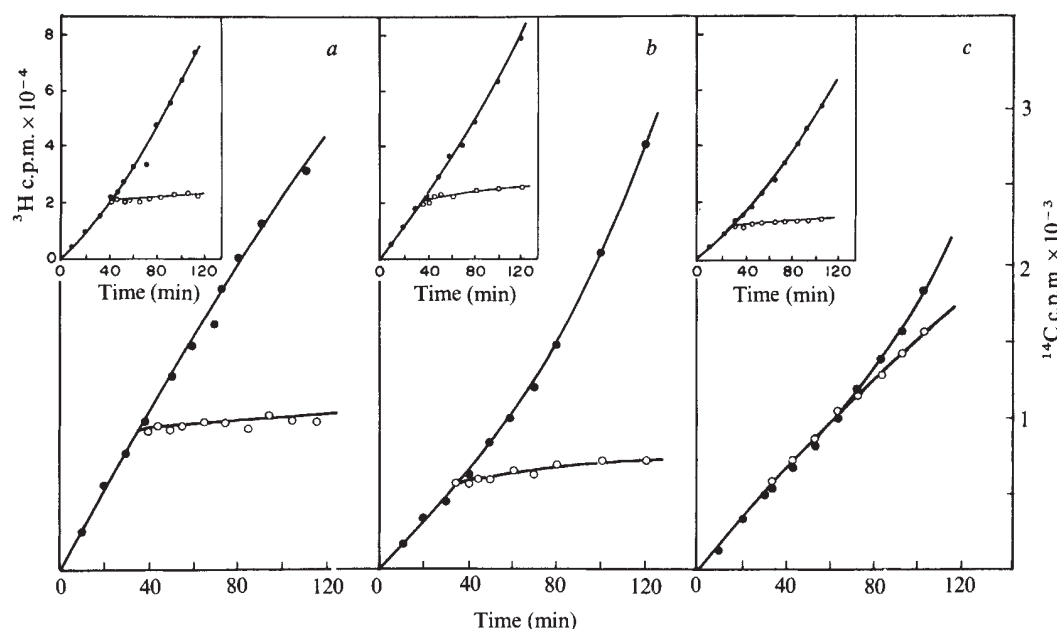
levels during amino acid deprivation, but does not do so when deprived of histidine and perhaps other amino acids. Figures 1 and 2 show that in *S. typhimurium* the extent of the ppGpp response depends on the required amino acid, as in *E. coli*⁴.

Figure 3a, b shows that deprivation of histidine causes the arrest of protein and stable RNA synthesis to the same extent in strains TA997 and TA1001, which suggests that strain TA1001 has inherited a functional *relA*⁺ gene from its TA997 parent. To prove that this arrest of stable RNA synthesis in strain TA1001 is dependent on *relA*⁺ function, the effect of histidine deprivation on stable RNA synthesis was determined in strain PD2, a *relA* derivative of TA1001 constructed by transduction. Figure 3 shows that in this strain, stable RNA synthesis is under relaxed control, thus confirming that strain TA1001 harbours a *relA*⁺ gene and demonstrating that the arrest of stable RNA synthesis described in Fig. 3b is indeed attributable to stringent control. The level of ppGpp does not change when strain PD2 is deprived of histidine. Furthermore, no change in the ppGpp level is observed when serine deprivation is induced by serine hydroxamate addition—confirmation that the *relA* allele is present in this strain.

Strains TA997 and TA1001 are isogenic with the exception of the small segment of bacterial genome carrying the co-transducible markers *purF*, *hisT* and *aroC*. The fact that ppGpp metabolism is normal in TA997 strongly suggests that the defect present in TA1001 harbours one or more additional mutations close to *hisT*. The *hisT* gene product catalyses the pseudouridine modification in the anticodon region of the many tRNAs that contain pseudouridine in this region⁸. Mutations in the *hisT* gene alter the regulation of several amino acid biosynthetic pathways⁸, and histidine tRNA derived from a *hisT* mutant is defective in translation⁹. Thus, it is possible that the alteration in ppGpp and pppGpp metabolism in strain TA1001 is another consequence of the subnormal pseudouridylation of histidine tRNA. The three amino acids which produce a normal response (Fig. 2) in TA1001 are cognate to tRNA species all or most of which have no pseudouridine in the anticodon region, and are therefore not affected by the *hisT* mutation. Note that one of a different class of histidine regulatory mutants, *hisU*, is altered in the control of the accumulation of stable RNA during carbon and energy source transitions¹⁰.

We do not know whether the abnormal response of TA1001 to histidine deprivation is due to a block in the production of

Fig. 3 RNA and protein synthesis in strains TA997, TA1001 and PD2 deprived of histidine. Bacteria were grown to a concentration of 4×10^8 cells per ml in low-phosphate medium, and ^{14}C -uridine ($0.01 \mu\text{Ci ml}^{-1}$, $5 \mu\text{g ml}^{-1}$) and ^3H -isoleucine ($1 \mu\text{Ci ml}^{-1}$, $3.5 \mu\text{g ml}^{-1}$) were added. Half the culture was filtered after 30 min and resuspended in medium lacking histidine but containing the labelled precursors at the same specific activities as before. Samples were taken up in 5% TCA, filtered on Millipore filters and counted in a Packard Tri-Carb scintillation counter. Large graphs show incorporation into RNA; insets show incorporation into protein. a, TA997; b, TA1001; c, PD2. ●, Not deprived; ○, deprived. PD2 (*hisD2655*, *hisT1514*, *relA1::Tn10*) was constructed by transducing *relA1::Tn10* into TA1001.



ppGpp or to an increase in its degradation. The main conclusion that can be drawn from the above observations is that an increase in the intracellular concentration of ppGpp is not a necessary condition for stringent control of the net synthesis of stable RNA. This leads to one of the two following conclusions: either ppGpp has no causal role in stringent control of RNA accumulation, or inhibition by ppGpp is not the only mechanism, but exists alongside and competes with at least one other mechanism also dependent on the *relA*⁺ gene. In case of failure of the ppGpp mechanism, the putative accessory mechanisms would become totally responsible for stringent control. With respect to the control of RNA accumulation, the latter hypothesis finds some support in several studies which have shown that a considerable fraction of the decrease in net synthesis of stable RNA can be attributed to degradation of the RNA^{11,12}. Thus, it may be that in TA1001, stringent control of RNA synthesis is totally degradational.

The results obtained with strain TA1001 also make it rather unlikely that the effector molecule in stringent control could be a derivative of ppGpp, such as guanosine 5'-diphosphate-3'-

monophosphate (ppGp), as has been suggested³. The role of the guanosine polyphosphates in bacterial physiology is now, more than ever, an open question which a thorough examination of the properties of strain TA1001 may elucidate.

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Nuclear sodium and potassium

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Na^+ and K^+ influence gene expression^{1–3}, and it is important to know the concentrations and chemical activities of these cations in the cell nucleus and to understand their control. However, measurements are hampered by the small size of nuclei, and also because (1) the steep Na^+ and K^+ activity gradients across cell membranes can cause experimental manipulations to provoke rapid (~ 0.1 s half-time for a $10\ \mu\text{m}$ cell) artefactual cation redistributions; (2) intracellular activities cannot be directly inferred from concentrations because Na^+ , K^+ and water exist partly in bound form. We circumvent these difficulties by using a giant cell (amphibian oocyte), cryogenic methods to limit diffusion, and an artificial intracellular reference phase^{4,5}. Century *et al.*⁶ found K^+ more concentrated in the oocyte nucleus than in cytoplasm, and the reverse for Na^+ . Our data for the *Desmognathus o. ochrophaeus* (salamander) oocyte are similar: the nucleus contains $30 \pm 3\ \mu\text{equiv}\ \text{Na}^+$ and $144 \pm 3\ \mu\text{equiv}\ \text{K}^+$ per ml H_2O ; the cytoplasm contains $87 \pm 2\ \mu\text{equiv}\ \text{Na}^+$ and $87 \pm 2\ \mu\text{equiv}\ \text{K}^+$ per ml H_2O (means $\pm$ s.e.m.). These data could imply that the nucleus actively transports Na^+ and K^+ , but strong evidence suggests otherwise: the nuclear envelope, with pores of effective radius $45\ \text{\AA}$ (ref. 7), is too permeable to maintain small solute concentration gradients^{8–10}, and microelectrodes measure equal nuclear and cytoplasmic cation activities^{11–13}. Here, we confirm and extend these findings, showing that nucleus/cytoplasm cation concentration differences are due to (1) partial exclusion of diffusive Na^+ and K^+ by cytoplasm and (2) differential Na^+ and K^+ binding by nucleus and cytoplasm.

Oocytes were microinjected at 34°C with 50–100 nl of 14% gelatin sol, sometimes containing ^3H -sucrose. The gelatin, which displaces a volume of cytoplasm, was gelled by immersing the oocyte in iced Ringer's solution for 5 min. Subsequent incubation was at 5°C . The gelled gelatin or reference phase (RP) excludes organelles and structural elements, and its water comes to diffusional equilibrium with intracellular solutes^{4,5}. Oocytes were then frozen at -160°C in dichlorodifluoro-

methane, and stored in liquid nitrogen. Nucleus, RP and cytoplasm were isolated at -45°C by ultralow-temperature microdissection, weighed, dried and reweighed to determine water content, and extracted in water by boiling^{6,14}. Cation concentrations were determined by atomic absorption spectroscopy and ^3H -sucrose by liquid scintillation counting.

Intracellular ^3H -sucrose does not distribute uniformly at equilibrium^{5,15}, nuclear and RP concentrations being equal, but about three times that of the cytoplasm (Fig. 1). Because sucrose distributes uniformly when gelatin is dialysed against saline¹⁵, we deduce that the nucleus/cytoplasm asymmetry is not due to nuclear binding, but to sucrose's reduced solubility in, or exclusion from, cytoplasmic water.

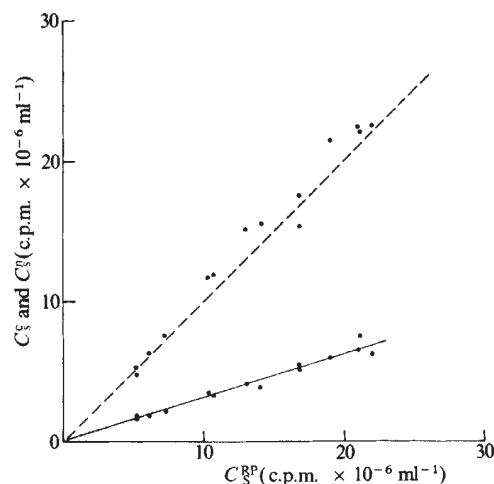


Fig. 1 Nucleus/RP (dashed line) and cytoplasm/RP (solid line) represent concentrations of ^3H -sucrose (C_s). Oocytes were manually dissected from ovaries in ice-cold Ringer's solution containing (mM) 104.5 NaCl, 2.5 KCl, 1.2 MgSO_4 , 6.6 NaHCO_3 , 1.2 Na_2HPO_4 , 2.0 NaH_2PO_4 and 0.7 CaCl_2 (pH 7.2), and injected with gelatin containing ^3H -sucrose, incubated for 4–20 h, dissected by ultralow-temperature microdissection, and analysed as described in the text. C_c^s , C_n^s and C_{RP}^s are ^3H -sucrose concentrations (per ml H_2O) in cytoplasm, nucleus and RP, respectively. The least-squares line for cytoplasm/RP data is $C_c^s = 0.31 C_{RP}^s + 0.01$ ($\times 10^6$ c.p.m. ml^{-1}). The dashed line is isomolar, $C_n^s = C_{RP}^s$; the least-squares line determined for the nucleus/RP data (not shown) is $C_n^s = 1.04 C_{RP}^s + 0.15$ ($\times 10^6$ c.p.m. ml^{-1}).

Figure 2 compares Na^+ and K^+ concentrations, C_{Na} and C_{K} , in RP, nucleus (n) and cytoplasm (c). $C_{\text{Na}}^{\text{RP}}$ and C_{K}^{RP} are reciprocally related, their sum constant at $\sim 151 \mu\text{equiv ml}^{-1}$ (Fig. 2a). There is a clustering of cells with $C_{\text{K}}^{\text{RP}}/(C_{\text{Na}}^{\text{RP}} + C_{\text{K}}^{\text{RP}}) > 0.7$ (Fig. 2, inset). We consider these cells 'normal' because their cytoplasmic and nuclear cation concentrations resemble those of uninjected cells⁵. Typical of eukaryotic cells, Na^+ and K^+ activities (measured by the RP) in normal oocytes are, respectively, less and greater than those of the Ringer's (Fig. 2 legend). $C_{\text{K}}^{\text{RP}}/(C_{\text{Na}}^{\text{RP}} + C_{\text{K}}^{\text{RP}}) < 0.7$ results from damage during microinjection and experimental manipulation, but these values allow isothermal analysis of mechanisms. Nuclear data resemble RP data, but most nuclear points fall above the RP line (Fig. 2b). (In normal oocytes $C_{\text{Na}}^{\text{n}} + C_{\text{K}}^{\text{n}} = 171 \pm 4 \mu\text{equiv ml}^{-1}$.) Also, when $C_{\text{Na}}^{\text{n}} > 100 \mu\text{equiv ml}^{-1}$, Na^+ increase is not stoichiometrically compensated by K^+ loss, as in the RP. C_{K}^{n} and C_{Na}^{n} are also reciprocally related, but C_{K}^{n} is always $< 105 \mu\text{equiv ml}^{-1}$ and $C_{\text{Na}}^{\text{n}} > 70 \mu\text{equiv ml}^{-1}$ (Fig. 2c). Obviously, cytoplasm, RP and nucleus handle cations differently.

Cytoplasmic and RP Na^+ are not isomolar (Fig. 3a, open circles). As gelatin/saline dialysis isotherms are isomolar⁴, these data demonstrate the existence of two cytoplasmic Na^+ fractions, one shared with the RP and therefore diffusive, and the other restricted to the cytoplasm, or bound. The concentrations of diffusive and bound Na^+ (relative to total cytoplasmic water) are denoted by $C_{\text{Na}}^{\text{c1}}$ and $C_{\text{Na}}^{\text{c2}}$, respectively, so that $C_{\text{Na}}^{\text{c}} = C_{\text{Na}}^{\text{c1}} + C_{\text{Na}}^{\text{c2}}$. Like sucrose, diffusive Na^+ has reduced solubility in cytoplasm, the cytoplasm/RP diffusive Na^+ distribution coefficient (q_{Na}) being 0.45 (the slope in Fig. 3a). The intercept, $75 \mu\text{equiv ml}^{-1}$, is the saturation concentration of bound cytoplasmic Na^+ , $\text{max-}C_{\text{Na}}^{\text{c2}}$.

The nucleus behaves similarly to the RP (Fig. 3b). At low Na^+ concentrations nuclear and RP data are superimposable, showing that nucleus and cytoplasm share only diffusive Na^+ and that the nucleus interacts with Na^+ as though it were an RP or ordinary saline solution (Fig. 3b). When $C_{\text{Na}}^{\text{n}} > 100 \mu\text{equiv ml}^{-1}$, increase in RP (diffusive) Na^+ is accompanied by a larger increase in nuclear concentration. Perhaps the nucleus contains macromolecular charge groups which are unmasked when exposed to $\text{Na}^+ > 100 \mu\text{equiv ml}^{-1}$ (refs 8, 16).

Cytoplasmic K^+ , like Na^+ , consists of two fractions, a diffusive fraction, of concentration C_{K}^{c1} , shared with the RP, and an unshared, nondiffusive fraction, of concentration C_{K}^{c2} (Fig. 4a). The cytoplasm/RP distribution coefficient for diffusive K^+ , q_{K} , is 0.36 (slope, Fig. 4a). The intercept at $41 \mu\text{equiv ml}^{-1}$ is the saturation concentration of bound cytoplasmic K^+ , $\text{max-}C_{\text{K}}^{\text{c2}}$. RP and nucleus concentrations are similar, differing only in a small ($19 \mu\text{equiv K}^+ \text{ml}^{-1}$) invariant nuclear excess (Fig. 4b). Apparently, nuclear water acts like ordinary or RP water as solvent for K^+ , but in addition the nucleus contains $19 \mu\text{equiv ml}^{-1}$ of bound K^+ .

A previous study of *Rana pipiens* oocytes incubated in $^{42}\text{K}^+$ -Ringer's¹⁷ revealed two cytoplasmic K^+ fractions indistinguishable from those demonstrated here. Exchange of the fast isotopic fraction, present in both nucleus and cytoplasm, was limited by cell-membrane permeability. The second fraction, restricted to cytoplasm, exchanged too slowly to be measured. As short-term incubations suggested that $^{42}\text{K}^+$ reflects the distribution of diffusive K^+ , we tested this by incubating RP oocytes for 3–4 h in $^{42}\text{K}^+$ -Ringer's, and found that the cytoplasm/RP concentration ratio of $^{42}\text{K}^+$, q_{K}^* , was 0.36, identical to q_{K} from the K^+ isotherm. Hence, $^{42}\text{K}^+$ exchange with cytoplasmic bound K^+ is negligible, and diffusive K^+ , like sucrose and Na^+ , is more concentrated in the RP than in cytoplasm. Also, the $^{42}\text{K}^+$ nucleus/RP ratio for normal oocytes was 1.14 ± 0.03 , agreeing with Fig. 4b in demonstrating nondiffusive nuclear K^+ . However, bound nuclear K^+ exchanges completely with $^{42}\text{K}^+$ (nucleus/RP specific activities ratio = 1.00 ± 0.03) in < 3 h, while cytoplasmic exchange is extremely slow.

At equilibrium, the RP—an ordinary water phase with no membrane—and the nucleus are essentially isomolar for diffusive Na^+ , K^+ and sucrose, demonstrating that the nucleus

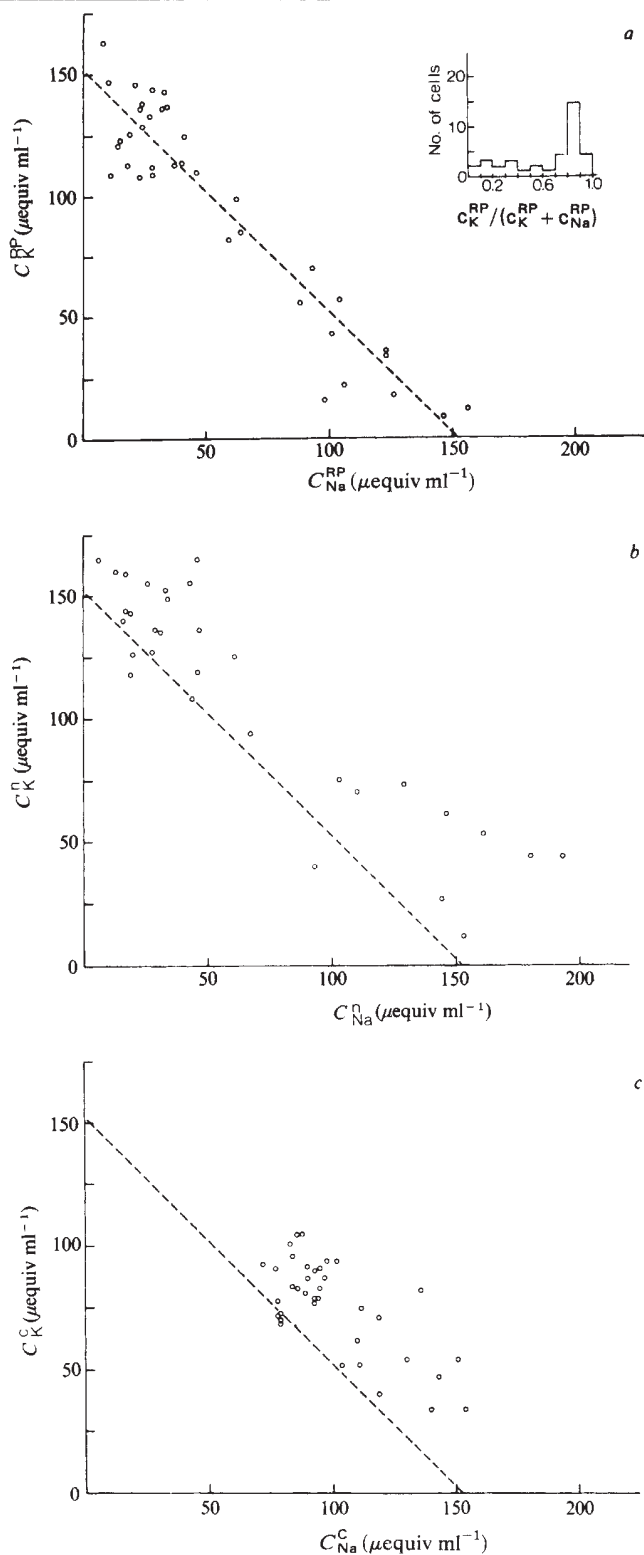


Fig. 2 Concentrations of K^+ and Na^+ (C_{K} and C_{Na}) in RP, nucleus and cytoplasm. *a*, RP data for cells fitted by the least-squares line, $C_{\text{K}}^{\text{RP}} = -C_{\text{Na}}^{\text{RP}} + 151 \mu\text{equiv ml}^{-1}$. (Inset, frequency distribution of cells as a function of fractional RP K^+ content.) Equilibrium dialysis shows that RP gelatin has ordinary water solvent properties. Hence, intracellular solute activities (a_i) can be determined from $a_i = C_i^{\text{RP}} \gamma_i^0$, in which $\gamma_i^0 \sim 0.77$ is the activity coefficient in salines of comparable concentrations⁵. In normal oocytes ($C_{\text{K}}^{\text{RP}}/(C_{\text{Na}}^{\text{RP}} + C_{\text{K}}^{\text{RP}}) > 0.7$) cytoplasmic and nuclear activities are $99 \mu\text{equiv K}^+ \text{ml}^{-1}$ and $18 \mu\text{equiv Na}^+ \text{ml}^{-1}$, compared with Ringer's $2 \mu\text{equiv K}^+ \text{ml}^{-1}$ and $89 \mu\text{equiv Na}^+ \text{ml}^{-1}$. *b*, *c*, Data for nucleus and cytoplasm, respectively. Least-squares line from RP data (dashed) is shown for comparison.

does not actively transport cations or sucrose. Furthermore, the RP directly reflects effective cytoplasmic free concentrations and hence chemical activities⁴. Except for a small amount of binding, the same is true for the nucleus, which also behaves toward microsolutes like an ordinary aqueous phase embedded in a complex cytoplasm. Nuclear solute control can therefore only be understood when we know exactly how cytoplasm differs from simple aqueous solutions. Phenomenologically, two factors are identifiable: exclusion and binding. Cytoplasmic exclusion of solute *i* is indexed by q_i , the cytoplasm/nucleus or cytoplasm/RP equilibrium distribution coefficient of the diffusive form. If binding is negligible, as with sucrose and $^{42}\text{K}^+$ at short exchange times, the coefficient is equal to C_i^c/C_i^n or C_i^c/C_i^{RP} . When binding is significant, q_i is the slope of the cytoplasm/RP isotherm asymptote. Present determinations are q_s (sucrose) = 0.31 (Fig. 1), $q_K^* = 0.36$, $q_K = 0.36$ (Fig. 4a), and $q_{\text{Na}} = 0.45$ (Fig. 3a). Bound cytoplasmic concentrations are also

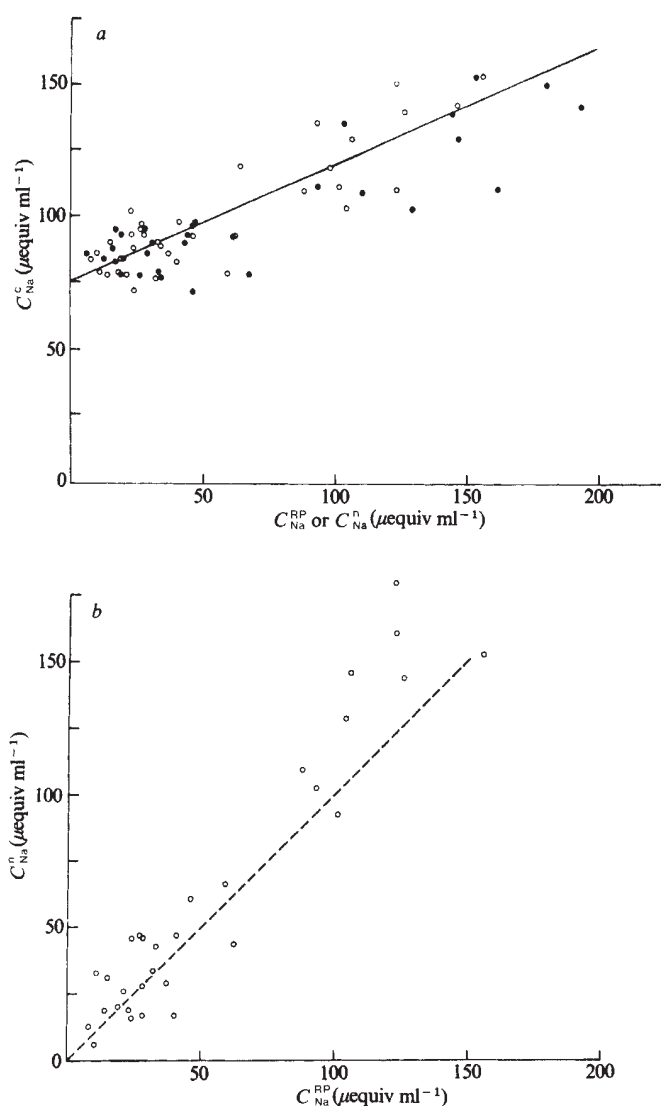


Fig. 3 Sodium isothermal relations. *a*, Cytoplasmic Na^+ concentration, C_{Na}^c , versus RP Na^+ concentration, $C_{\text{Na}}^{\text{RP}}$ (open circles), and nuclear Na^+ concentration, C_{Na}^n (closed circles). The line is the least-squares line for the cytoplasm/RP data, $C_{\text{Na}}^c = 0.45 C_{\text{Na}}^{\text{RP}} + 75 \mu\text{equiv ml}^{-1}$. *b*, C_{Na}^n versus $C_{\text{Na}}^{\text{RP}}$. The dashed line is isomolar, the least-squares line (not shown) is $C_{\text{Na}}^n = C_{\text{Na}}^{\text{RP}} + 4 \mu\text{equiv ml}^{-1}$.

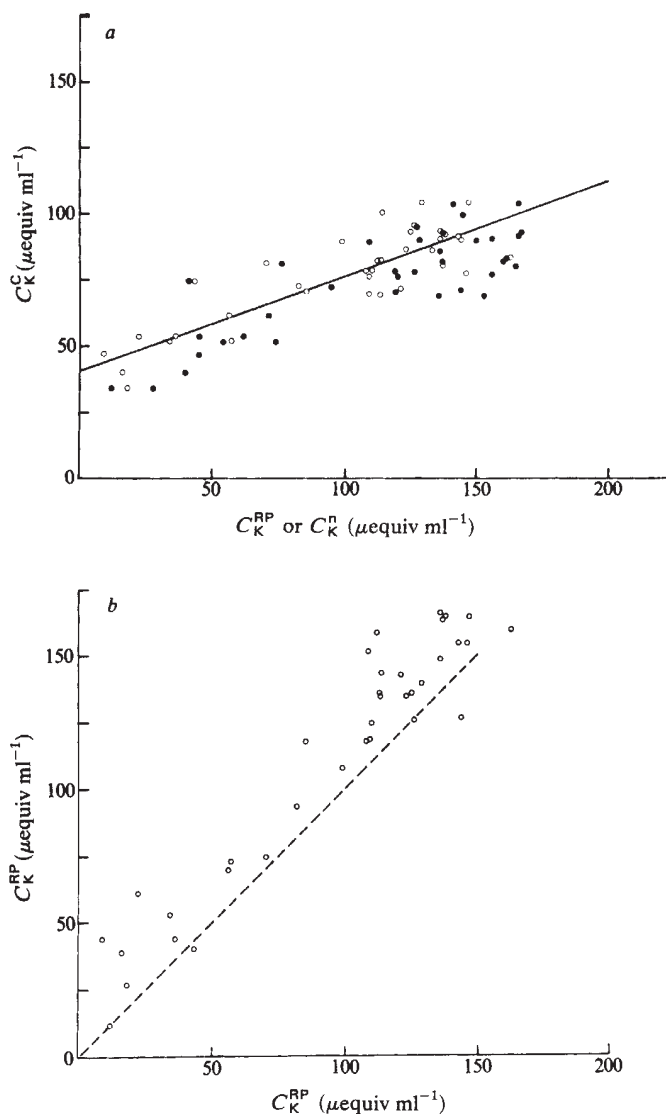


Fig. 4 Potassium isothermal relations. *a*, Cytoplasmic K^+ concentration, C_K^c , versus the RP K^+ concentration, C_K^{RP} (open circles); the least-squares line is $C_K^c = 0.36 C_K^{\text{RP}} + 41 \mu\text{equiv ml}^{-1}$. C_K^c compared with nuclear K^+ concentration, C_K^n (closed circles); the least-squares line (not shown) is $C_K^c = 0.34 C_K^n + 38 \mu\text{equiv ml}^{-1}$. *b*, C_K^{RP} versus C_K^c . The dashed line is isomolar; the least-squares line (not shown) fitting the data points is $C_K^{\text{RP}} = C_K^c + 19 \mu\text{equiv ml}^{-1}$.

determined from cytoplasm/RP isotherms; at saturation, C_{Na}^{c2} and C_K^{c2} are 75 and 41 $\mu\text{equiv ml}^{-1}$, respectively. (There is evidence that when C_i^{RP} is very low, the isotherms are nonlinear⁵, implying that some bound cations are in mass action equilibrium with diffusive cations.) Changes in the affinities and/or capacities of cytoplasmic binding and changes in cytoplasmic solvent capacity must be considered as possible mechanisms for transient modulation of the baseline level of nuclear solute activities maintained by the cell membrane¹⁸.

Cytoplasmic exclusion and binding can be approached through two paradigms. One assumes that most cell water is saline-like, but is partially in organelles or vesicles, surrounded by membranes with restricted permeabilities and/or active transport properties¹⁹. The second hypothesizes decreased solubility due to structuring of cell water^{5,18}. Vesicle and structured water mechanisms are not mutually exclusive. Oocyte cytoplasm contains abundant candidates (ground cytoplasm, endoplasmic reticulum, lysosomes, mitochondria, yolk plate-

lets) to account for exclusion and binding within either or both paradigms.

Exclusion and binding seem to be widely distributed properties of cells. The low nucleus (or RP)/cytoplasm Na^+ ratio which indicates that the diffusive/total Na^+ ratio in oocyte cytoplasm is low, is paralleled by Na^+ -specific electrode determinations of low activity/concentration ratios ('apparent' activity coefficients) not only in oocytes^{20,21}, but also in neurones²², epithelia^{12,23} and muscle^{22,24-26}. Similarly, the high nucleus (or RP)/cytoplasm K^+ ratio of oocytes is paralleled by high activity/concentration ratios determined with K^+ electrodes in oocytes²¹ and other cells^{5,27}. Deviation of cytoplasmic activity coefficients from those of ordinary salines is direct evidence of solute binding or exclusion. However, because binding and exclusion have opposing influences on activity/concentration ratios, intracellular activity coefficients resembling those in salines do not prove that cytoplasm is a simple water phase²⁷. Similarly, one cannot conclude that nucleus and cytoplasm are necessarily physicochemically similar if their Na^+ or K^+ concentration ratios are close to unity.

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Structural homologies between prealbumin, gastrointestinal prohormones and other proteins

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Prealbumin has unusual properties for a plasma protein. It is an unglycosylated small protein composed of four identical 127-residue polypeptide chains, and although generally thought to be of hepatic origin¹, its site of synthesis has not been shown. However, its amino acid sequence²⁻⁴, conformation and molecular interactions⁵⁻⁸ are known. Prealbumin has a role in retinol⁹ and thyroxine¹⁰ transport, and in the metabolic regulation mediated by thyroid hormones^{11,12}. A thymic hormone-like activity has also been reported¹³. Prealbumin-like immunoreactivity has been demonstrated in the α cells of the pancreas¹⁴ and in endocrine cells of the small intestine (T.P., B.J., B. Sandstedt and A.C., unpublished results). A relationship with some activity of the gastrointestinal tract is therefore possible. Here we show sequence similarities between prealbumin and the glucagon-secretin type of gastrointestinal hormones, as well as between prealbumin and retinol-binding protein. Possible evolutionary links with prohormones and other functions in a new protein family are demonstrated—these may explain the unusual properties of prealbumin.

The primary structures of mammalian glucagon, secretin, vasoactive intestinal peptide (summarized in ref. 15), gastric inhibitory peptide¹⁶ and the C-terminal part of the possible glucagon prohormone¹⁷, glicentin¹⁸, were compared with the structure of human plasma prealbumin. The hormones are related, forming a family¹⁵. All possible hormone alignments

along prealbumin were tested using a computer; randomly generated sequences with identical total compositions were similarly compared. By this method, the probability of identities shared between the actual structures belonging to the distribution of background similarities could be calculated.

With the exception of gastric inhibitory peptide, all these hormones give only one alignment with prealbumin at a low probability level, and this alignment is the same for the four hormones, starting at position 90 in prealbumin (see Fig. 1). The probabilities, single fits and identical positions suggest that the hormones align uniquely with the C-terminal part of prealbumin. Gastric inhibitory peptide (Fig. 1) falls outside this pattern, but it is very different from other members of the hormone group¹⁵.

To test the significance of the similarities further, comparisons were made with retinol-binding protein¹⁹ (functionally related to prealbumin) and with horse liver alcohol dehydrogenase (no known relationship to any of these polypeptides). In the case of retinol-binding protein, all sequence identities to the hormones were insignificant (fivefold greater chance occurrence than with prealbumin, Fig. 1), and alignments were not unique but multiple (for three hormones) or starting at different positions (two hormones). For alcohol dehydrogenase, probability values were variable, and the alignments were again multiple (three hormones) or single but different (two hormones). Thus, the hormone comparisons with retinol-binding protein and alcohol dehydrogenase support the significance of the similarities in Fig. 1.

The C-terminal location of the similarity as well as the size of prealbumin agree with the idea of an homology between prealbumin and a glucagon prohormone. Glicentin¹⁸ has the glucagon sequence in the C-terminal region and a size similar to that of the prealbumin subunit¹⁸. The same features apply to the somatostatin precursor²⁰.

These independent protein data support the patterns of sequence similarities in Fig. 1. The prealbumin homology to a prohormone of the glucagon family is unexpected and resembles other super-families¹⁵ detected by sequence comparisons, for example, lysozyme-lactalbumin²¹, haptoglobin-serine proteases²² or ovalbumin-antiproteases²³. It is even possible that prealbumin has retained a prohormone function in addition to its known interactions with retinol-binding protein and thyroxine. It exhibits thymic hormone-like activity¹³ but independent of this or other activities, the structural homology with

	90	100	110	120	127	Identities with prealbumin	Chance probability	Unique alignment
PRE	HAEVVF TAND SG PRRYTIAALLSPYSYSTTAVVTNPKE							
SECR	HSDGTFT SEL S RRL R DSARLQRLQLGLV					6	0.015	+
VIP	HS DA VFT DN Y TRL R KQMAVKKYLNSILN					6	0.010	+
GLIC	HS QGT FT SD Y S KYLD S RR A QDFVQWLMNTKRNNIA					7	0.013	+
GLUC	HS QGT FT SD Y S KYLD S RR A QDFVQWLMNT					6	0.010	+
GIP	Y A E GT F ISD Y S IAMD K IRQQDFVNWLLAQKGKSDWKHNITQ					5	0.134	-

Fig. 1 Results of sequence comparisons between human prealbumin (PRE), pig secretin (SECR), pig and bovine vasoactive intestinal peptide (VIP), part of pig glicentin (GLIC), pig and human glucagon (GLUC) and pig gastric inhibitory peptide (GIP). Amino acid residues are shown by one-letter codes, with similarities between prealbumin and any other peptide in bold type. Positional numbers refer to prealbumin. Probability values represent the chance occurrence of alignment of each peptide independent of the others, as determined by the program described. Unique alignment indicates that no other fit of the peptides along prealbumin gives the same number of identities.

gastrointestinal hormones explains the tissue localization of prealbumin.

The structures of prealbumin and retinol-binding protein¹⁹ were compared using all possible combinations of 30-residue segments. Three pairs of similarities (well below the 0.01 random probability level) were distinguished from all other pairs (above the 0.01 level), and could also be correlated with each other (see Fig. 2). One pair suggests an internal repetition in retinol-binding protein, and the other two pairs relate each of these two repetitions to one segment of prealbumin. The positional alignments of the three pairs detected are similar for the mutually independent comparisons, only deviating a little in the case of the prealbumin segment.

The repetitive region in retinol-binding protein involves 11 identities in 43 residues (26% identity; $P = 0.0005$ chance occurrence, Fig. 2), giving values as distinctive as those for the established protein connections between yeast and horse alcohol dehydrogenases. Furthermore, the two segments in retinol-binding protein detected from internal comparisons (positions 17–59 and 58–100, Fig. 2) agree almost exactly with those (positions 15–58 and 60–97) revealed by the comparison with prealbumin. The similarities to prealbumin involve a shift in positional numbers, but are still at the 0.005 probability level (Fig. 2). If not merely reflecting conformational similarities, such shifted numbers could indicate gaps, as shown for another peptide comparison (H.J., V.M. and M.P., unpublished results).

The relative positions of the segments are summarized in Fig. 3a. The region marked 'common' in prealbumin has similarities that are less significant than those in the other regions. However, all common regions are compatible with an ancestral connection between prealbumin and retinol-binding protein, and a partial duplication in the latter. If significant, these regions correspond to the most conserved and common function of both proteins. One such region may be the area involved in the binding interactions between prealbumin and retinol-binding protein.

An ancestral connection between the two proteins is also supported by the size of the subunits. A duplication of the common region (consisting of ~40 residues) in retinol-binding protein, and some shifts due to gaps, make the corresponding ancestors similar in length. The present size difference is 55 residues, with prealbumin the shorter subunit, as anticipated by such a scheme.

The position of the two segments in the conformation of the prealbumin subunit is shown in Fig. 3b. Both regions cover large areas and cannot be ascribed to single functions. However, in the tetramer, parts of the 'hormone' regions limit the thyroxine binding site (Fig. 3b). The association between a primary structure homologous to one hormone and a tertiary structure binding another would, if ancestrally significant, support the idea that receptors and their ligands have a common evolutionary origin^{24–26}. In the divergence from the ancestor, prealbumin would then have become a member of the receptor line rather than of the prohormone line. An ancestral connection between nuclear receptors and prealbumin has also been inferred⁷ from the presence of a DNA binding site in prealbumin. The start of the hormone region (positions 90–96) forms the bottom of the tetramer's cylindrical impression which constitutes the DNA binding site (Fig. 3b).

The central parts of the common region in the prealbumin chain are superficial in the tetramer (Fig. 3b), and could therefore be involved in the binding of the retinol-binding protein. However, it is not known whether this is true, and it will be of interest to see if the sequence similarities between the two proteins correspond to folding similarities. If so, this part of retinol-binding protein would contain six β strands, as each common region in prealbumin (Fig. 2) has three β strands⁸. Anyway, known conformational arrangements in prealbumin fit the scheme suggested from the observed homologies. Opposite arrangements of the hormone and common segments would not have provided similar support.

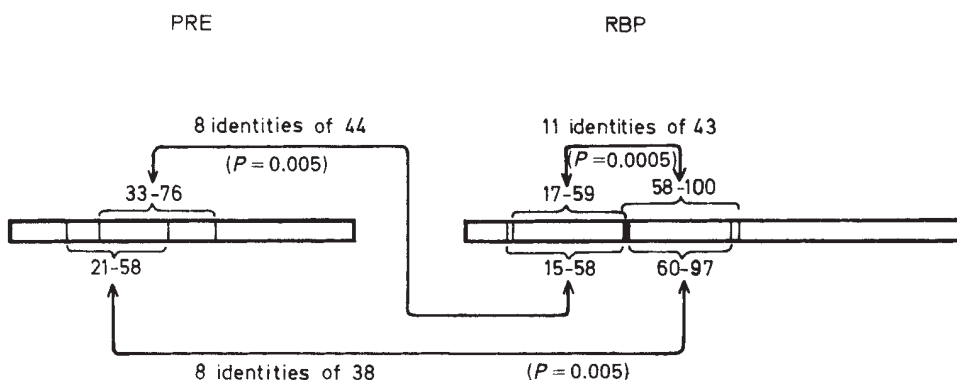


Fig. 2 Schematic representation of the three single pairs (double arrows) of sequence similarities detected in all possible combinations of segment comparisons between and within the subunits of prealbumin (PRE) and retinol-binding protein (RBP). Polypeptides (bars) and similarities (with end positions indicated) are drawn to scale. Probability values (P) represent the chance occurrence.

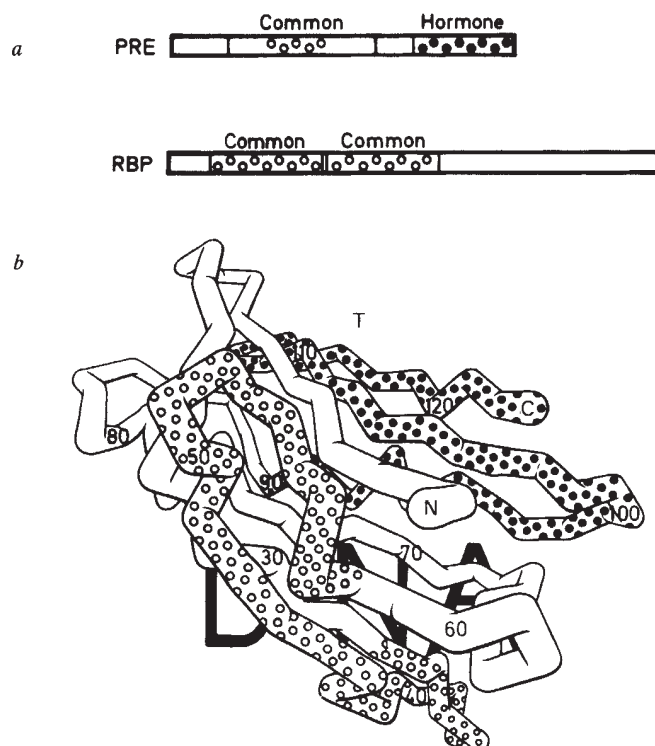


Fig. 3 *a*, Schematic representation of the regions discussed in prealbumin (PRE) and retinol-binding protein (RBP). 'Hormone' is the region homologous to the glucagon-secretin group of peptides; the other three homologous regions are indicated by 'Common'. *b*, Illustration of the regions discussed in a drawing of the conformation of the prealbumin subunit. Filled dots indicate the 'Hormone' region and empty dots the central part of the 'Common' region (positions in Fig. 2). In the native tetramer, a second subunit is above the one shown, with the binding site for thyroxine (indicated by T) in between, and another subunit is behind the one shown with the DNA binding site (indicated by DNA) in between. This drawing was made with the help of Fig. 3b of ref. 8.

The sequence similarities observed for prealbumin suggest evolutionary connections with gastrointestinal prohormones and retinol-binding protein. The data are compatible with the known properties of the prohormones, the conformation and binding interactions of prealbumin, and with previous ideas about the origins of receptors. They also suggest another superfamily of related proteins, indicating complex endocrine functions while helping to explain the unusual properties of prealbumin.

We thank Hans Nilsson for the drawing, Dr Steven Feinmark for linguistic help, and the Swedish MRC and Cancer Society for support (projects 13X-3532, 13X-1010 and 620, respectively). *Note added in proof:* Another comparison involving retinol-binding protein has just been reported²⁷ in which an internal although different duplication was observed (only second in present comparison). The discrepancies illustrate difficulties in comparisons and may question conclusions, but could also indicate further overlapping duplications.

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Structure of the binuclear iron complex in metazidohaemerythrin from *Themiste dyscritum* at 2.2 Å resolution

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Haemerythrin and myohaemerythrin are non-haem iron proteins utilized by several marine invertebrates in transporting and storing oxygen. Haemerythrin is oligomeric [usually octameric, but also trimeric^{1,2}, and tetrameric and dimeric (C. A. Appleby, personal communication)] whereas myohaemerythrin is a monomeric analogue of the haemerythrin subunit. Physicochemical investigations³⁻⁵ of these molecules have shown that (1) each subunit binds two iron atoms by means of amino acid side chains, (2) one molecule of O₂ is bound to each binuclear iron centre, and (3) the iron atoms are in the +3 oxidation state in oxy and met forms of the proteins and +2 in deoxy. Models have been proposed for the complexes in haemerythrin and myohaemerythrin on the basis of crystallographic studies of the proteins. A μ -oxo bridged model (Fig. 1a) has been proposed from the results of a crystallographic analysis of metazidomyohaemerythrin from *Themiste zostericola*⁶. Based on a similar analysis of methydroxohaemerythrin from *Themiste dyscritum*, previously referred to as metazidohaemerythrin⁷, we proposed a confacial bi-octahedron model with no atom identified as a μ -oxo bridge (Fig. 1b)⁸. Previously⁹, we stated that "at least one of the models is incorrect". In fact, neither completely accounts for the electron density from the present 2.2 Å resolution difference map of the complex and surrounding electron density of metazidohaemerythrin from *T. dyscritum*.

Crystals of metazidohaemerythrin were obtained by direct reaction of oxyhaemerythrin with 0.01 M NaN₃ and subsequent crystallization by dialysis against low ionic strength buffer. The space group is P4, $a = b = 86.61(2)$ Å, $c = 80.90(2)$ Å. The 2.2-Å data set, ~60,000 reflections, including Friedel pairs, was collected by the $\omega/2\theta$ step-scan method¹⁰ from three metazidohaemerythrin crystals (27,131 reflections with $I > 2\sigma(I)$, 31,532 total reflections). Both empirical absorption¹¹ and deterioration corrections were applied.

A previously refined model of methydroxohaemerythrin¹² served as the initial model for this study. All atoms associated with the iron complex were removed, including those beyond the β -carbon atoms in the side chains of the amino acids shown as ligands in Fig. 1b. For the model lacking the complex, $R (= \sum |F_{o, \text{azide}}| - |F_c|) / \sum |F_{o, \text{azide}}|$ was 0.288. This model was sub-

jected to two cycles of restrained least-squares refinement¹³ to relieve the calculated phases of bias from the input structure, reducing R to 0.246. A difference electron density map calculated with coefficients $|F_{o,azide}| - |F_c|$ and calculated phases provided an essentially unbiased view of the electron density associated with the complexes in each of the four crystallographically independent subunits. Features of the difference density, such as resolution of the iron atoms, resolution across the ring of tyrosine 109 and resolution across the six-membered rings formed by the carboxylate groups, the iron atoms and the bridging atom, indicate the high quality of the map.

A straightforward interpretation of the difference density map for metazidohaemerythrin leads to the model shown in Fig. 2 having features in common with each of the earlier ones. The complex consists of two octahedrally coordinated iron atoms bridged by two carboxylate groups and an oxygen atom. The other ligands are three histidine side chains for one iron and two histidine side chains and the azide ion for the other. There is no density connecting tyrosine 109 (114 in myohaemerythrin) and the iron complex. The azide ion clearly binds to only one iron atom, rather than bridging the two as suggested by our earlier, low-resolution metazido study¹⁴. The observed mode of binding is as predicted by Gay and Solomon in interpreting their polarized, single-crystal spectroscopic results¹⁵. The coordination portrayed in Fig. 2 has not been observed in any other iron complex or metalloprotein, although it is similar to several small molecule iron carboxylate structures¹⁶.

The model proposed for the complex in metazidomyohaemerythrin and shown in Fig. 1a has the bridging oxygen atom (a feature supported by spectroscopic and magnetic studies), but lacks the bridging carboxylate groups. On the other hand, the model proposed for the complex in methydroxo-haemerythrin shown in Fig. 1b has bridging aspartic and glutamic acid side chains, the details of which were left unspecified. The model also has an additional bridging group which was assumed to be a water molecule or a hydroxide ion. Crystallographic refinement of the methydroxo-haemerythrin form at 2.0 Å resolution had previously led to a more detailed view of the complex showing that the carboxylate groups bridge the two iron atoms with separate oxygen atoms on each iron. Tyrosine 109 was found to be too far from the nearest iron atom to be a ligand although it remained in the same eclipsed position observed earlier⁸. It is not coordinated either directly or indirectly through a water molecule, implying that one of the iron atoms is penta-coordinate. The resulting model is essentially the same as the present metazido model shown in Fig. 2, but lacks a ligand at the azide binding site.

A major question posed by the metazido structure and the bridging oxygen is whether the bridging atom in methydroxo is a single oxygen atom or some other species such as hydroxide. Another is whether there is any evidence for a hydroxide ion being bound at the azide binding site. In answer to the first question, we note that it is impossible in the electron density map at the present resolution to differentiate between a single

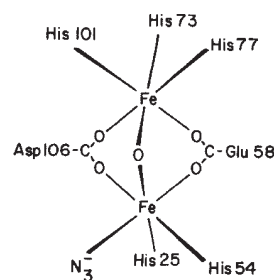


Fig. 2 The iron complex in metazido-haemerythrin from *T. dyscritum*.

oxygen atom, a hydroxide ion or a water molecule. The only ligand density not accounted for by amino acid side chains in methydroxo-haemerythrin was at the bridging position. In accordance with the generally held opinion at the time that in met derivatives, small molecule ligands such as OH^- , N_3^- , SCN^- and H_2O were bound directly to the binuclear iron complex¹⁷⁻¹⁹, we considered the bridging density to be the small ligand. However, as the bridging group in the metazido structure is present in the same location, it seems to be a common feature of the complex and is probably a μ -oxo atom as indicated by spectroscopic and magnetic evidence.

To answer the second question and to make a more definitive comparison of the methydroxo and metazido forms of haemerythrin, we have restricted the resolution of the methydroxo data to 2.2 Å and treated it in the same way as the metazido data. We find small peaks in the difference density at the azide binding sites in all four subunits in the asymmetric unit although they are no larger than some noise peaks found in the open solvent regions of the crystal. The low occupancy of the sites indicates that only a fraction of the molecules have a sixth (as yet unidentified) ligand. Thus, the major form of the complex in the conditions pertaining in the crystals has an unexpected penta-coordinate iron atom.

The present model clarifies the contradictory crystallographic models previously proposed for the complexes in haemerythrin and myohaemerythrin. The difficulty arose in part because the met derivatives had been assumed to be similar, but significant structural differences clearly exist between at least two of them. Differences are observed in the spectra of the various states of these molecules, and high-resolution studies of other derivatives of met, oxy and deoxy forms of haemerythrin may allow significant correlations between the spectroscopic and structural results.

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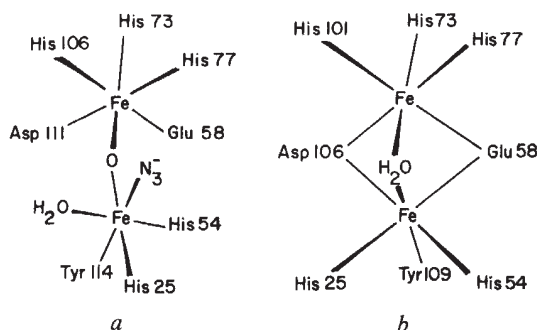


Fig. 1 Earlier models proposed for the binuclear iron complex in (a) metazidomyohaemerythrin from *T. zostericola*⁶ and (b) methydroxo-haemerythrin from *T. dyscritum*⁸ (drawn from Fig. 4, ref. 9).

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MATTERS ARISING

Si, Al ordering
in Linde A zeolite

ALTHOUGH we congratulate Bursill *et al.*¹ on their excellent high-resolution images of zeolite Linde A, we wish to question some of their interpretation—in particular the evidence on Si, Al ordering.

For a Linde A zeolite with Si/Al ratio of exactly 1, regular alternation of Si and Al leads inexorably to a super-cell with $a = 2.4$ nm and space group $Fm\bar{3}c$ from the pseudo-cell with $a = 1.2$ nm and space group $Pm\bar{3}m$ for fully disordered Si and Al. Although many structure determinations have been made with the pseudo-cell, the superstructure diffractions are easily observable on long-exposure X-ray photographs. Gramlich and Meier² obtained a satisfactory refinement of the superstructure of Zeolite A in which alternate tetrahedra had dimensions appropriate for occupancy at least mainly by Si and by Al. Recent refinements of dehydrated K-exchanged zeolite A (ref. 3) and dehydrated zeolite A (ref. 4) (the as-synthesized Na variety) were made to test the concept of zero-coordination, and satisfactory refinements were obtained of the superstructure. All these X-ray diffraction data, and much unpublished data, cannot be explained by the ordering model proposed by Engelhardt *et al.*⁵ on the basis of high-resolution magic-angle spinning ^{29}Si NMR measurement. This model yields a unit cell with $a = 1.2$ nm and space group $Pm\bar{3}$, and can be ruled out immediately by the X-ray diffraction data which require a cell with $a = 2.4$ nm. In the following, note that zeolite A has strong alternation of Si and Al on the tetrahedral nodes of the framework, and that only a small percentage of nodes are not surrounded by four atoms of the opposite type.

Pluth and Smith³ found evidence from detailed electron microprobe analyses of A crystals made by Charnell's method that the Si/Al ratio is >1 (~ 1.03). This was supported by population estimates from published^{3,4} and unpublished X-ray measurements which indicated less than 12 exchangeable monovalent cations in the pseudo-cell (for example 11.5 Na atoms in dehydrated zeolite A (ref. 4)). Such deviation from the ideal value must cause some Si atoms to be surrounded by less than four Al atoms. Silica-rich regions might have a different stability than regions with Si/Al equal to unity, and Bursill *et al.* might find it useful to measure the Si/Al ratio of their crystals.

Although Pluth and Smith refined their structures with the 2.4-nm cell and space group $Fm\bar{3}c$, they and earlier workers found several observed diffractions which violated the c glide plane. Further measurements are now in progress to dis-

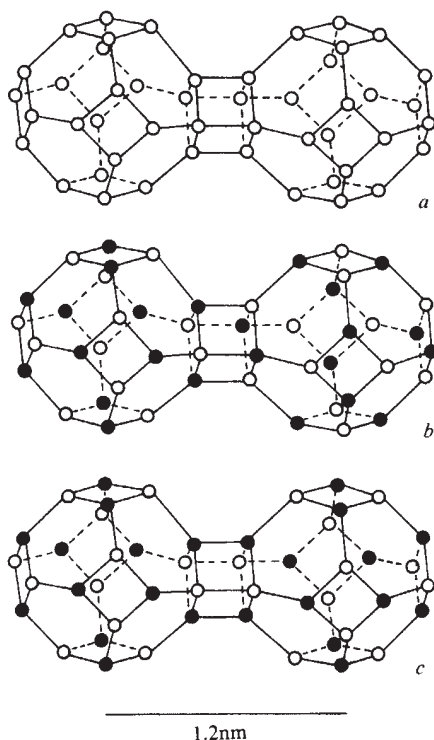


Fig. 1 Three ordering schemes for zeolite A. *a*, Complete disorder of tetrahedrally coordinated cations would give a 1.2-nm cell repeat with space group $Pm\bar{3}m$. *b*, Regular alternation of Si and Al requires 2.4 nm and $Fm\bar{3}c$. *c*, The isometric cell for the model of Engelhardt *et al.*⁵ has a 1.2-nm repeat and $Pm\bar{3}$; note the absence of a mirror plane in $\{110\}$ directions.

cover the source of these violations. Note that these diffractions are weak and might result from the exchangeable cations and not from the Si and Al atoms. In the dehydrated K-exchanged- and Na-A varieties, some exchangeable cations have a low occupancy of sites on the walls of cavities, and local distortions of the framework can be expected. Such distortions must be considered as a potential cause of space group violations.

The ^{29}Si NMR results were interpreted by Engelhardt *et al.*⁵ in terms of an ordering scheme in which each Si is surrounded by three Al and one Si, and each Al by three Si and one Al (Fig. 1). This ordering scheme gives an isometric cell with $a = 1.2$ nm and space group $Pm\bar{3}$, in contrast with 1.2 nm ($Pm\bar{3}m$) for complete disorder and 2.4 nm ($Fm\bar{3}c$) for regular alternation of Si and Al. The 3:1 ordering scheme is not compatible with the 2.4-nm cell repeat found consistently in long-exposure X-ray photographs, and can be ruled out except for small regions. Simple comparison of NMR data from one zeolite with another is obviously unreliable, and further interpretation is needed of the

data in ref. 5. The observation by Bursill *et al.* of hkl (all odd) reflections with $h + l = 2n$ does not confirm "the recent criticism of space group $Fm\bar{3}c$ ". This class of diffractions obeys the F centred lattice deduced by the X-ray crystallographers. Violations of the condition for the c glide plane (l must be even in hhl and $0kl$) are needed to cast doubt on $Fm\bar{3}c$ (for example, observation of 111; 3,3,17; 7,7,23; 13,13,11; 15,15,15 for dehydrated A ref. 4).

We suggest that Linde A zeolite has strong alternation of Si and Al atoms, and that only local regions deviate therefrom. The new refinement of dehydrated A zeolite⁴ could be used to make a computer-simulated image for comparison with Fig. 1 of ref. 1: qualitatively a good match is likely, thereby indicating extraction of water by the pre-pumping procedure¹. We look forward to more important observations of zeolites by electron microscopy, and suggest that existing X-ray diffraction data can provide a fundamental basis for interpretation.

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BURSILL ET AL. REPLY—We thank Smith and Pluth for their comments but we are not convinced by their arguments. Recent experimental results reaffirm that Na-A zeolites possess an aluminosilicate ordering scheme, in which each Si ion is connected through oxygen bridges to three Al ions and one Si ion, and each Al ion to three Si and one Al; that is 3:1, rather than 4:0, ordering prevails.

Our reasons for disputing the accepted space group $Fm\bar{3}c$ rests partly on some of the X-ray results of Smith and Pluth^{1,2} but chiefly on results from three other, independent techniques: electron diffraction^{3–5}, high-resolution magic-angle spinning ^{29}Si NMR^{6–8}, and neutron diffraction⁹. Weak superlattice reflections appear readily in electron diffraction patterns, requiring exposure times of ~ 1 s. enabling many hundreds of crystals

to be studied. We established that our preparations are homogeneous, with no evidence for local variations in intensity of weak reflections, and that doubling is observed in all zones when it is expected for a 24.6-Å unit cell. In particular *hhl* (inadvertently printed as *hkl* in ref. 3) reflections satisfying $h + l = 2n$ were observed. As it is not easy to assign unambiguously space groups using electron diffraction alone⁴ we have sought further information concerning space group assignment. Surprisingly, rather cogent evidence that the space group is not $Fm\bar{3}c$ comes from Smith and Pluth who measured 111; 3,3,17; 7,7,23; 13,13,11 and 15,15,15 intensities for dehydrated Na-A (ref. 1) and 999; 31,1,1, 21,21, 9 for dehydrated K-A (ref. 2). All of these reflections contradict the *c*-glide operation of $Fm\bar{3}c$. Smith and Pluth suggest that these reflections arise from displacements of cations and/or framework atoms, dismissing the NMR results⁶⁻⁸, which strongly implicate 3:1 ordering, on the grounds "that simple comparison of NMR data from one zeolite sample with another is obviously unreliable" and that the model proposed^{6,7} does not lead to a 24.6-Å cell. The NMR results are especially significant as careful calibrations of the chemical shifts, using known aluminosilicates as standards⁸, show the technique is extremely sensitive to the Si-O-Si (Al) coordination. The technique yields results for bulk specimens and merits at least the respect accorded to a crystal structure determination on one or two carefully selected X-ray specimens. We have pointed out⁴ that the model proposed by Engelhardt *et al.*^{6,7}, based on 3:1 ordering, yields an isometric cell with $a = 12.3$ Å and space group $Pm\bar{3}$, and suggested that further NMR and diffraction studies, encompassing a range of Si/Al ratios, should prove instructive, as cell doubling may arise to accommodate excess Al (or Si)^{4,5}.

New data on Si, Al ordering in zeolites have emerged from neutron diffraction studies, carried out in collaboration with A. K. Cheetham. Three dehydrated samples, covering a range of Si/Al ratios, were studied. Our analysis shows that the neutron diffraction patterns cannot be indexed using space group $Fm\bar{3}c$. However, refinement of the structure is proceeding satisfactorily using a space group having rhombohedral symmetry. Analysis of the geometrically possible Si, Al distributions required by this space group, and which maintain 3:1 ordering, required by the NMR results, and which give 24.6-Å cells, as required by the X-ray and electron diffraction results, leads to a new description of the structure, details of which are to be given elsewhere⁹.

We believe, therefore, that the 4:0 ordering scheme advocated by Smith and Pluth for Linde A must be viewed with reservation, at least for the bulk of Na-A preparations. Application of the above-

mentioned range of techniques to other zeolites, where novel ordering schemes may be uncovered, is indicated.

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Modern adaptations in orang-utans?

SMITH AND PILBEAM'S¹ letter, proposing that orang-utan sex differences—body size and certain features of dental and craniofacial morphology—cannot be explained by the species' modern adaptations and thus are 'remnants' of a more terrestrial Pliocene pattern, states that sexual selection among orang-utans is not clearly indicated by field observations, citing Horr² to the effect that "direct evidence for male competition in the form of dominance or aggressive encounters is limited".

The Tanjung Puting study³, now in its ninth year, indicates that all adult male-male encounters involve either aggression or avoidance as do most contacts between sub-adult and adult males. Aggression is the invariable response when two adult males encounter one another in the presence of an oestrous female. The large size of orang-utan males seems to be the result of sexual selection as there is intense male-male competition for oestrous females and female selection of males during consortships^{3,4}. In addition, several workers have found orang-utan males and females using differential proportions of resources³⁻⁵ and at least one³ has argued for an ecological separation of the sexes which may help explain sex differences as well.

Several features of orang-utan dental and craniofacial morphology are at variance with those characteristic of other arboreal frugivores—this can be explained by the fact that orang-utans spend an average of 40% of their feeding time on bark, young leaves, insects and other foods³. During some months orang-utans spend less than 20% of their

foraging time consuming fruit. One instance of meat-eating among a wild orang-utan population has just been reported (Sugardjito, personal communication). Furthermore, some of the fruits (for example *Mezzettia leptopoda*) eaten by orang-utans are extremely hard. Chimpanzees use tools to open very hard fruits⁶, but orang-utans spend hours opening hard nuts with their molars, which may account for their exceptionally thick molar enamel.

Finally, note that orang-utans can be quite terrestrial, bringing them closer to chimpanzees in this regard than is usually realized³. At Tanjung Puting adult males averaged 66 min per day on the ground with one male spending up to 6 h per day on the forest floor. A clear understanding of modern orang-utan adaptation suffices to explain most morphological features cited by Smith and Pilbeam as indicative of a more terrestrial Pliocene pattern. In regards to the fossil evidence for terrestrial patterns, we need post-cranial remains of ancestral orang-utans before we can discuss such patterns.

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SMITH AND PILBEAM REPLY—The data reported by Galdikas are interesting, but the implications for our argument are unclear. What is directly known of ancestral orang-utans (their teeth and something of their distribution) is suggestive of a more terrestrial niche than that usually described for the extant species. Whether or not features of the extant animal support this hypothesis is secondary because one would expect the modern species to be more or less adapted to what it does. Also, whether or not the behaviours observed by Galdikas are sufficient to explain the morphological features in question is entirely speculative.

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BOOK REVIEWS

Biology and social science: wedding or rape?

Edmund Leach

THIS book comes so close to being a parody of the genre to which it belongs that I have had difficulty in believing that it is not intended as an academic hoax. Edward Wilson has made important contributions to the study of the social behaviour of insects and has achieved a wider notoriety as popularizer of the concept of sociobiology. He is also the author of a notably undistinguished book entitled *On Human Nature* (Harvard University Press, 1978); Lumsden is "a physicist who has extended his research into theoretical biology". The authors jointly claim an "abiding respect for social theory, the neurosciences and psychology". I cannot comment on their knowledge of the neurosciences and psychology but, on the evidence here presented, they appear to have no understanding of social theory whatsoever.

The basic proposition of the book stems from a belief that "the advance of men over chimpanzees and other higher primates was attained during an exceptionally rapid burst of evolution . . . over a period of approximately three million years". This alleged rapidity of a postulated unilinear evolutionary progress is held to be causally associated with a special kind of feedback reinforcement between the genetic constitution of human beings and their culture (here renamed "euculture"). This latter theme is expounded with the aid of a massive battery of diagrams, graphs and sophisticated-looking algebraic equations.

From my own doubtless prejudiced but none the less professional point of view, the whole of this elaborate apparatus is devoid of meaning because it assumes that human culture can be broken down into clusters of traits (which the authors call "culturgens") which can then be counted and subjected to statistical analysis.

The notion of culturgens is defined at some length and is summarized as "equivalent to the artifact type employed in archaeology (by D. L. Clarke)". From then on the whole argument presupposes that culturgens are readily identifiable entities. With very few exceptions, contemporary social and cultural anthropologists are unanimous in holding that human culture cannot be broken down into units of this or any other kind. The late D. L. Clarke, who was once my pupil, would certainly have rejected the idea.

However, empirical evidence plays only a secondary role in the authors' presentation. It is only drawn upon to

Genes, Mind, and Culture: The Co-evolutionary Process. By Charles J. Lumsden and Edward O. Wilson. Pp.512. ISBN 0-674-34475-8. (Harvard University Press: 1981.) \$20. The book will be published in the UK in July, price £12.

illustrate points which they already judge to be self-evident. A typical example of the way this is done comes on p.6:

From the time of the beginnings of a materials-based culture in the ancestral *Australopithecus* to the Upper Palaeolithic era of modern *Homo sapiens* the brain tripled in size. The cranial capacity of *Australopithecus* was 400–500 cc., comparable to that of the chimpanzee and gorilla. Two million years later *Homo erectus* had attained a capacity of 1000cc. Another million years saw an increase to 1400–1700 cc. in Neanderthal man and 900–2000 cc. in modern *Homo sapiens*.

Yet the authors must certainly know: (i) that it is far from clear that any of the Australopithecine species so far identified was directly ancestral to *Homo*; (ii) that *Australopithecus*, though man-like in shape, was very small, so it is not surprising that it had a small head; (iii) that the evidence available concerning the early hominids and the early varieties of *Homo* is statistically miniscule (in particular, as the authors' figures indicate, it is quite possible that all the *Homo* skulls fall within the range found in modern man); (iv) that since, in modern man, there is no correlation at all between cranial capacity and manifested intellectual capacity the size of our heads has no bearing whatever on the theme of the book which is "evolutionary advance" (whatever that means).

The "parody of science" aspect of the presentation can be illustrated from Chapter II at the end of which we are told that:

Epigenesis is the total process of interaction between genes and environment during the course of development with the genes being expressed through epigenetic rules.

This explains the otherwise baffling opening sentences which read:

The key element in the theory of gene-culture coevolution is the role of the epigenetic rules in culturgens choice. These rules and the bias curves they generate can serve as the molecular units in postulational-deductive models of the social sciences.

If we dig through the jargon it turns out that all this really means is that man's

genetic endowments make it likely that some aspects of culture will be more elaborately developed than others. Thus visual and auditory discriminations, as manifested in speech and music and lexicon, are always more precise than those which depend upon taste and smell.

These platitudes about sensory perception are then extended into the field of custom and the concept of culturgens comes into its own. Once again the crass idiocy of the whole proceedings can only be illustrated by quotation:

Female puberty initiation rites originate most frequently in societies where a young girl continues to reside at least half the time in her mother's household (in other words where uxori-local or bilocal residence rules are in force), as well as in societies that depend heavily on the work of girls and young women to make a major contribution to sustenance activities.

Recognising that some culturgens are more derivative and communicative in function than others, it is possible to delineate what appear to be chains of causation connecting the principal habitat, economic strategy, and both primary and derivative cultural choices. A very reduced example is the following:

Habitat and economy	Principal culturgens	Derivative culturgens
Desert herders	→ Polygyny, patrilineality, patrilocality	→ Absence of formal female initiation ceremony

Just what this could have to do with genetics is hard to imagine, unless of course the authors suppose that uxori-local and patrilocality populations are racially distinct! But, for the record, it deserves note that the first half of the first paragraph is gibberish while the second half is such a let out that it would cover almost anything. "Female puberty initiation rites" is a broad category but the most drastic of the better known forms are those which involve clitoridectomy. These practices were traditionally confined to regions of East Africa where nomadic pastoralists with a more or less patrilineal/patrilocality/polygynous form of social organization are particularly prominent. It would be difficult to imagine a worse exemplification of the Lumsden-Wilson "chains of causation".

All the other examples of "gene-culture translation" which the authors mention (and they mention very few) are similarly defective. For example they claim that there is:

a nearly universal avoidance of marriage and full sexual relations between full brothers and sisters. The epigenetic rule appears well

established: a deep sexual inhibition develops between those who live in close domestic contact during the first six years of life.

But no such rule has been "well established". On the contrary there is good evidence that in modern Western society sibling incest is quite common and there is no good reason to suppose that it is rare elsewhere. Moreover, in direct contradiction to what is asserted by Lumsden and Wilson, there is unambiguous evidence that monogamous marriage was widely practised in ordinary peasant families in Egypt during the first three centuries of our era and very probably for millennia before that. It does not require "epigenetic rules" to explain why marriages of this sort are usually forbidden; the proscription follows directly from the social theory for which Lumsden and Wilson have such abiding respect but so little understanding.

The human species is not genetically uniform but the variations seem to be minor. No correlation has yet been established between known genetic variations and morphological variations. Equally there is no clear correlation between morphological variations (the so-called "racial types"), cultural variations and/or modes of subsistence. But equally there are no cultural universals except at the level of such generalizations as "all human beings have speech", "all human societies distinguish between domestic space and non-domestic space".

If epigenetic rules have had consequences for human evolution, it is difficult to see what sort of rules they might have been. On this central feature of the authors' argument the phoney mathematical apparatus which characterizes this text offers no illumination.

The book ends by setting a programme for social science:

The decomposition of social behaviour into objective functional units, the discovery of new and sometimes surprising epigenetic rules, the measure of human genetic diversity, the tracing of the microevolution of the features of behavioral epigenesis, the paleobiological reconstruction of the origins of culture, the retrodiction [*sic*] of ethnography and connection of the results to new and more powerful covering laws in economics and sociology, perhaps even a sighting down the world-tube of possible future histories—these are activities that will come increasingly to occupy the social sciences as the links between biology and the study of culture are more powerfully forged.

I will readily agree that the findings of biology and of social science need to be compatible if either are to rate as science. But what is called for is mutual respect on both sides. The jargon loaded take-over bid proposed by Lumsden and Wilson is just bunk. We need a marriage — not a rape. □

Edmund Leach is a Fellow of King's College, Cambridge.

Marine mineralogy

Roger G. Burns

Underwater Minerals. By D. S. Cronan. Pp.362. ISBN 0-12-197480-4. (Academic: 1980.) £24.80, \$57.50.

THE publication of J. L. Mero's *The Mineral Resources of the Sea* by Elsevier 16 years ago drew attention to the vast mineral deposits on the seafloor. Subsequent events — notably expanded oceanographic research, improved mineral beneficiation and extraction processes, real and threatened embargoes of industrial minerals and fuels, expansion of coastal state jurisdiction to the 200 mile limit, and legal ramifications of the United Nations Law of the Sea Conferences towards deep-sea mining of manganese nodules — have heightened the public's awareness of the potential of submarine mineral resources.

The appearance of Dr Cronan's book, therefore, is particularly significant at this time of rapidly accumulating information from modern oceanographic and marine geochemical research. In one concise volume, Cronan surveys several types of underwater mineral deposits, relates their origins to current global plate tectonic models, describes submarine exploration methods and explores some of the consequences of mining marine minerals. He calls upon his own experiences and observations from years of oceanographic cruises and laboratory research. The text focuses on various types of mineral deposits mainly located beneath the oceans; the extraction of minerals from seawater by modern processes or from ancient evaporite deposits is not discussed.

Chapters in Cronan's book are devoted to placers and aggregates, including deposits of gold, platinum, diamond, cassiterite (tin oxide), ilmenite and other heavy minerals mined near continental margins; phosphorites on continental shelves; miscellaneous authigenic minerals such as zeolites and barite; manganese nodules and encrustations from deep sea floors, marginal seas and lakes; metalliferous sediments related to volcanic activity along spreading centres, mid-ocean ridge crests and fracture zones, island arcs and submarine volcanoes; and sub-surface deposits such as ore and coal seams adjacent to continents, and sulphide mineralization in modern or uplifted (ophiolite) ocean floor basalts. The concluding three chapters relate marine mineral deposits to recent theories of plate

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To help scientists cope with the complex problem of information retrieval, *The Use of Scientific Information Services* by R. Davidge and E.R. Wooding describes the use of information services in libraries, and contains a listing of reference sources. The report is available from The Library, Royal Holloway College, Egham Hill, Egham, Surrey TW20 0EX, UK, price £2.

tectonics and ocean evolution, describe methods for exploring underwater mineral deposits, and review some of the factors leading to the mining, and consequences resulting from exploitation, of seafloor minerals.

On the whole, the author does an excellent job in surveying such a broad subject. He references, perhaps uncritically, a wide spectrum of published papers including many of his own noteworthy contributions, so that a beginner is well informed about each topic while a researcher is guided to significant, albeit familiar, publications. Obviously, in one 362-page book, there is inevitable lack of

depth of coverage in some topics. However, recent readily available specialist texts and monographs complement Cronan's book by providing detailed coverage of marine manganese nodule deposits and other specific marine minerals. The author has achieved his objective to write an up-to-date textbook of interest to undergraduate and post-graduate students, civil servants concerned with marine affairs and people generally interested in the marine environment. □

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Records of the past

John Currey

Skeletal Growth of Aquatic Organisms: Biological Records of Environmental Change. Topics in Geobiology, Vol.1. Edited by D.C.Rhoads and R.A. Lutz. Pp.750. ISBN 0-306-40259-9. (Plenum: 1980.) \$47.50, £29.93.

THIS book explores what we can learn from the skeletal structure of aquatic organisms about the environment in which they lived, and how the environment affected their lives. The editors point out that until recently much of the drive for such studies has come from palaeontologists interested in reconstructing palaeoenvironments, but that now ecologists are also becoming interested in how the recent past can be revealed by skeletons.

The editors have clearly put much thought into the organization of the book, and have given their contributors clear briefs. As a result, they have produced a book that is admirable for the beginner and also for old hands.

There are 16 chapters and 12 appendices. Twelve of the main chapters concern molluscs; they range from a general overview of shell growth and form in bivalves to particular examples of the effect of the environment on shell structure of *Mercenaria*, and the recognition of developmental types from larval shell morphology. The balance in this mollusc section seems right; the major emphasis is on molluscs as a whole, and how the environment influences shell growth, yet there are some examples of how these principles apply to particular populations or species. In all the contributions it is reiterated that while the environment has marked effects on the shell, the interpretation of, for instance, check lines, changes in mineralogy, or oxygen isotope ratio, needs great care. The message, however, is not that the subject is so difficult that only people who have worked on it for 20 years should attempt to publish but rather that it is one in which both the fascinations and the pitfalls are densely distributed.

After the contributions on molluscs, the four on other groups (barnacles, corals, fish sagittae and polychaete jaws) seem meagre, though they are all interesting. The editors defend themselves against this criticism by stating that most work on environmental influences on skeletons is being done on molluscs. This may be true, but if so it is a shame.

A particularly good feature is the appendices. They are really instructive and will enable people, after a few mishaps no doubt, to prepare thin sections or acetate peels, or to distinguish crossed foliated structure from ordinary crossed lamellar structure. There are also useful chapters and appendices that deal with demographic and statistical matters.

One of the few unsatisfactory features of this book is that the subject of the effect of

Stylish distillation for petrologists

M. P. Atherton

Metamorphic Petrology: Mineralogical, Field and Tectonic Aspects. 2nd Edition. By Francis J. Turner. Pp.524. ISBN 0-07-065501-4. (McGraw-Hill: 1981.) £25.95, \$40.05.

THE second edition of Francis Turner's book is a considerable modification and updating of a volume, first published in 1968, which has been a milestone on the path towards our understanding of metamorphic rocks. It has increased in size from 403 to 524 pages, and although many of the original diagrams have been kept, many new ones have been added. Nevertheless, even with these additions the stylish character of the book has been maintained. There are eleven chapters as opposed to eight in the first edition, and most of this change has involved a new division and expansion of the subject, rather than the addition of entirely new material. The references are now placed at the end of the book rather than at the end of each chapter, and lie next to an extensive author index. It is interesting to note that even though many new authors are cited, Eskola, Fyfe, Verhoogen, Goldschmidt and Tilley still dominate the references.

In spite of the fact that much fresh material and many new ideas have been introduced, the main thread of the original book is maintained, and is "still by way of Eskola's facies concept" which allows the mineralogical and field material to be most readily "perceived, marshalled and analysed". The new edition also follows the approach in two earlier books (*Metamorphic Reactions and Metamorphic Facies* by W.S. Fyfe, F.J. Turner and J. Verhoogen *Geol. Soc. Am. Mem.* 73, 1958; *Igneous and Metamorphic Petrology* by F.J. Turner and J. Verhoogen, McGraw-Hill, 1960) both classics of their time, in that they were the first to present an integrated experimental and thermodynamic approach in the field of petrology. Thus it contains ACF, AKF, AFM diagrams indicating the chemographic relations of mineral assemblages

defining the facies, PT diagrams, some simple thermodynamic theory and applications, and many maps of metamorphic areas showing the general geology and metamorphic zonal patterns.

In the account of these elements of metamorphism the book is excellent, and insofar as the aim of all metamorphic geologists as to be able to accurately plot the PT path of metamorphic rocks, the discussion on experimental data, Shreinemaker's diagrams and the use of thermodynamics is sensible and rightly critical — as is to be expected from such an experienced researcher and teacher whose working life has spanned the old and the new in metamorphic petrology. I particularly liked the addition of recent ideas on pore solutions, ionic reactions, Shreinemaker's analysis, geobarometry and geothermometry, burial metamorphism and new mineral equilibria.

However, if the aim of the metamorphic geologist is to plot the path of his rocks in PT space, I think it is also necessary to consider the textures in the rocks, to integrate them with the deformation history and with the sequence of reactions involving a fluid phase which occurred during metamorphism. This new edition includes little on these important aspects although, in fairness, much of the progress made in those areas, particularly in integrating them, was made after the first edition. Their incorporation into the present book would have involved a completely fresh approach, indeed a much longer book.

Despite these comments this new edition will, I am sure, continue to be influential and be much referred to. It should be read by every thinking student of metamorphism because it puts the subject into proper historical context and is the distillation of the lifetime views of a sharp-eyed, thoughtful and influential metamorphic petrologist. □

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selective predation on morphology is rather neglected. It has been known for some time that the intensity of predation may affect both intraspecific and interspecific variation of shell morphology. Some of this variation is very like that produced by environmental differences; indeed it is often a real problem, even in living populations, to disentangle the two. Furthermore, though some of the differences are the result of selection taking place within a generation, some have quite high heritable components. Tevesz and Carter do mention this problem briefly in their chapter on the unionaceans, but it should perhaps have been discussed more fully somewhere.

I noticed only a few examples of laxness in editing. For instance, to give what is essentially the same equation as

$$L_t = L_\infty (1 - e^{-kt})$$

and

$$N_t = N_{\max}(1 - ce^{-r_0 t})$$

in two different places is needlessly confusing. Also, in my copy (and therefore, presumably, in several hundred others) the pages in the appendices were hopelessly jumbled. This interesting, handsomely produced, well-illustrated book deserves many buyers — but they should all carefully check pages 640 onwards for continuity. □

John Currey is a Professor in the Biology Department at the University of York.

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Historians of science confront the hydra

Robert Fox

The Ferment of Knowledge: Studies in the Historiography of Eighteenth-century Science. Edited by G.S. Rousseau and Roy Porter. Pp. 500. ISBN 0-521-22599-X. (Cambridge University Press: 1980.) £25, \$39.50.

OVER the past decade, the historiography of eighteenth-century science has been totally recast. In the process, change and complexity have emerged as the leading characteristics of a period which, not so many years ago, was seen as a rather pedestrian interlude between the heady days of the scientific revolution and the expansive age of modern professional science that dawned in the first half of the nineteenth century. The reassessment owes much to the 13 contributors to this absorbing volume of essays. All of them have played a major role, in areas as diverse as industrial science and technology (treated here by Donald Cardwell), the chemical revolution (Maurice Crosland), the life sciences (Jacques Roger), "mathematical cosmography" (the title of Eric Forbes's contribution) and geology (Roy Porter). In *The Ferment of Knowledge*, however, their brief was to review the state of their particular art and to relate work in their field to larger cultural perspectives. "Strong interpretations" and "coherent analysis" were the objectives of the stock-taking, and it is by these lights that the book must be judged.

What, then, are the "strong interpretations" that emerge, and where are the new beacons? Remarkably, at least one tentative answer to these questions already seems possible. This is because the most obvious historiographical casualty of the 1970s is the very one that has traditionally dominated studies of science in the age of the Enlightenment: the notion of a unified, transcendent Newtonianism guiding confrontations with nature either as an inspiration or as a target for criticism. John Heilbron's revisionism on this point emerges from his plea for a more detailed study of the practice, as opposed to the metaphysical context, of experimental physics in the eighteenth century: as he observes, the single epithet "Newtonian" is at best unhelpful when applied to a discipline which underwent complete redefinition between the 1720s and 1800 and which flourished not only in Britain but also in regions of the Continent, notably in Germany, where the writ of Newton barely ran. Simon Schaffer makes even greater play of the conflicting traditions in eighteenth-century natural philosophy: with engaging panache, he argues for an analysis that would see natural philosophy as an autonomous form of discourse informing, and being informed by, the practice of scientific investigation, without

ever being part of it. In this way, he may well make better sense not only of the many-headed hydra that scholars of the Newtonian tradition have had to wrestle with but also of anti-Newtonianism of the kind proposed by John Hutchinson in the realm of matter-theory.

Some of the nice problems of interaction raised by Schaffer also appear in Steven Shapin's sophisticated study of the "social uses" of science. In his quest for a "new contextualism", Shapin eschews straightforward notions of interaction between the discrete realms of scientific theory on the one hand and social context on the other. For Shapin, the realms are inseparable, with the result that the content of scientific ideas (and not, for example, merely their diffusion) can only be understood if we also understand the social significance they possessed in the minds of their creators. Such an approach has already yielded a fundamental reinterpretation of Boyle's Christianized corpuscularianism (in the work of J.R. Jacob), and a consideration of differing social uses may also be the key to one of the great conundrums of eighteenth-century historiography: the existence of what appear to have been competing Enlightenments, including both "radical" (the term is Margaret Jacob's) and High Church versions which repudiated Newton's carefully articulated theism.

The Ferment of Knowledge would have been welcome enough if it had merely reviewed a fruitful field of research; the editors and their well-chosen contributors have ensured that it fulfils that task admirably. But the book also says much, by implication, about the state of the history of science as a whole. Time and again, it demonstrates the unprecedented receptiveness of historians of science to work in other disciplines. The techniques of anthropology and modern social history make a welcome appearance (the latter being used to particularly good effect in W.F. Bynum's richly documented paper on medical care). Michael Foucault, too, flits across the stage, though to responses that range from the critically tolerant to the dismissive. The marriage between the history of science and the social sciences would appear to have been fully consummated, to the obvious benefit of a discipline which has striven long and hard to divest itself of an embarrassingly antiquarian past. The aptness of the term "ferment" when applied to recent developments in the history of science needs no underlining. □

Robert Fox is Reader in the History of Science at the University of Lancaster and President of the British Society for the History of Science. He is the author of a critical edition of Sadi Carnot's *Réflexions sur la puissance motrice du feu* (Vrin, Paris: 1978).